PHYSICAL CHEMISTRY

IN THE

SERVICE OF MEDICINE

SEVEN ADDRESSES

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AUTHOR'S PREFACE.

THE addresses which have been collected for the
first time in this volume and which were delivered in
the main as summaries of my own special investigations
concern themselves with the application of physical
chemistry to different fields in medicine as rendered
possible more particularly through advances in the
physics and chemistry, of organic colloids. In that
questions in physiology as well as pathology and
pharmacology are touched upon, it may perhaps be
hoped that different circles of medical men may be
interested in the problems discussed in this volume.
The translated addresses differ in only a few unimpor-
tant abbreviations from the original. The development
of the guiding thought common to all of them stands
out quite clearly. Its foundation is *the extensive
parallelism between the laws which govern changes in
the colloidal state in vitro and in the living organism.*
Even though the future may become acquainted with
many a new fact through which the questions discussed
in this volume may be made to appear in a different
light, it will scarcely be possible to belittle the fruitful-
ness of the methods described and the stimulating effect

of the results obtained through them. Since all the results that these methods can yield are as yet by no means attained it is hoped that the volume may be looked upon as a modest attempt to win friends capable of work in this still young field of labor.

<div style="text-align: right">WOLFGANG PAULI.</div>

VIENNA, May 1. 1906.

TRANSLATOR'S PREFACE.

It is hoped that the following translation of a few of Dr. Pauli's papers may render some of the work of this modest Viennese investigator already familiar to a large circle of American and English workers accessible to yet others. The fundamental character of the subjects touched upon by the author needs no comment. It is only hoped that the translation may not have lost too much of the spirit and the letter of the original German. The volume as a whole represents another stone in the structure of physical chemistry in the biological sciences; and while it is not the tendency of modern times to divide existing sciences or to create new ones, specialism is followed as a matter of necessity, so that it will not seem strange if in the near future we shall come to recognize as branches developing separately from the trunk which all these sciences have in common, a *physico-chemical physiology* and a *physico-chemical pathology*.

<div align="right">MARTIN H. FISCHER.</div>

OAKLAND, CALIFORNIA.

PREFATORY NOTE TO AMERICAN EDITION.

THE advance of medicine is so dependent upon progress in the fundamental sciences of physics, chemistry, and biology that he who will keep abreast of modern conceptions in physiology and pathology is compelled to be more or less conversant with theory and practice in the basal subjects. When one considers the phenomenal development in recent years, through the work especially of Willard Gibbs, van't Hoff, and Arrhenius, in the domain of what is designated physical chemistry, it is not surprising that attempts should have been made to apply the new knowledge gained to the clearing up of some of the problems which confront the physician. While the application of stoichiometrical methods in medicine and biology has led and is leading to fruitful results, it is from the utilization of the principles of the other great branch of physical chemistry, that which deals with energy-relations in chemical processes, that most is to be hoped; that many of the medical conceptions of the future are to be colored by the ideas of thermochemistry, electrochemistry, chemical kinetics, and chemical dynamics even those of us who are entirely untrained in these sciences are compelled to admit. The work already done on reaction-velocity, catalysis, equilibrium, viscosity, osmotic pressure, and electrolytic dissocia-

tion in the human and animal body may be regarded as an earnest-penny of greater good hereafter.

The new medicine will require a new preliminary training of its workers. A few investigators in biology and medicine have been wise enough to foresee the path which future inquiries must follow; we should be thankful that they have prepared themselves for the pioneer work of blazing the trail. Notable among these hardy explorers are some of our foremost American workers in physiology. Among European scientists, Dr. W. Pauli of Vienna stands out prominently as a representative of the forward movement. His researches in physiology and pharmacology have dealt almost entirely with problems in the solution of which the methods of physical chemistry have been applied. In his recent studies in colloidal chemistry he has been prying into and attempting to illuminate some of the darkest of the regions in which physiological chemists grope.

The American publishers of Dr. Pauli's papers have been fortunate in securing the services of Dr. Martin Fischer as translator. The experience he has gained by his personal researches in similar fields, and his familiarity with the bibliography of the whole subject, especially fit him for the task.

May Dr. Pauli's papers stimulate American students to further investigations where they are so much needed, and may he and they collect speedily for us a body of facts which we, as medical men, may utilize in the diagnosis of disease and the cure of human ills!

LEWELLYS F. BARKER.

BALTIMORE, Oct. 23, 1906.

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PHYSICAL CHEMISTRY IN THE SERVICE OF MEDICINE.

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1. On Physico-chemical Methods and Problems in Medicine.*

THE last decades have brought with them an amalgamation of two sciences,—physics and chemistry,—which have no doubt always had mutual relations, although formerly these were not so intimate or extensive as they are now.

⟋ This amalgamation was undoubtedly inaugurated through physics, and must be attributed primarily to the stimulus which brought with it the establishment of the laws of thermodynamics.

I cannot here sketch even briefly the development of thermodynamics. As is well known, the law of the conservation of energy as most clearly enunciated by MAYER forms its foundation. The remarkable experiments of JOULE next led to an exact determination

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* Über physikalisch-chemische Methoden und Probleme in der Medicin, Wien, 1900, M. Perles. Address delivered to the *K. k. Gesellschaft der Aerzte*, Vienna, November 10, 1899.

of the mechanical equivalent of heat, while through HELMHOLTZ was developed and executed the most extensive programme for the application of the law of energy to all subjects.

The penetrative analysis of thermodynamical phenomena by CLAUSIUS and THOMSON completed the subject with the establishment of the so-called chief laws of thermodynamics.

The transformations in energy in chemical reactions have in general two sources. As is well known every change in the state of aggregation is accompanied by either an absorption or an evolution of heat. Since changes in physical state often accompany a chemical reaction, these constitute therefore one of the sources of the transformations in energy accompanying this reaction.

A second is found in the chemical reaction itself. The synthesis or analysis of a substance is accompanied by a thermal change which may have either a positive or a negative value. To illustrate this we may cite the formation of a salt from an acid and a base with the development of the so-called heat of neutralization; or the decomposition of a salt into its components with a using up of electrical energy.

All these metamorphoses in energy constituted from the first a fruitful field for work, in which medicine also soon took part. While, however, the decrease in the potential energy of the foodstuffs in the metabolism of men and the higher animals constitutes one of the best developed chapters of medicine, calorimetric investigations of the culture media of bacteria are still lacking, and this in spite of the fact that this subject

promises the solution of an important problem, namely, the energy of growth.

Further relations between chemical constitution and physical properties were discovered by the new science, physical or theoretical chemistry.

Under this heading must be mentioned first of all the connection discovered between optical asymmetry (rotation of the plane of polarized light) and asymmetry in chemical composition. At almost the same time LE BEL and VAN'T HOFF discovered that all optically active substances which in the non-crystalline state rotate the plane of polarized light contain an asymmetric carbon atom, the four valencies of which are connected with four different radicles. If we imagine these four valencies connected with the corners of a tetrahedron, the four radicles may be grouped in two different ways and be symmetrical. The development of this idea, which constitutes the foundation of stereochemistry, has been very fruitful.

The doctrine of the asymmetrical carbon atom is destined to play an important rôle in biological problems also, for such essential constituents of protoplasm as the proteins and many carbohydrates must, to correspond with their optical activity, contain such an asymmetrical carbon atom.

The connection between physical changes in state and chemical constitution was early indicated by the regularity with which bodies of the aliphatic series affect the boiling-point. More recently a connection between color and the position of certain groups in the molecule known as chromophores has been discovered. Similar conditions exist in the case of fluorescence which is

connected with the existence of fluorophore radicles, and in the case of antipyretics the effect of which is intimately associated with their chemical constitution.

The modern theory of solution as harmoniously enlarged through VAN'T HOFF's conception of the gas-like condition of the dissolved particles, and ARRHENIUS's teaching that electrolytes—salts, acids, and bases—dissociate upon solution into their constituent ions, has also found extensive scientific application to many subjects including medicine.

Following the establishment of these fundamental facts physical chemistry has developed as an independent science with numerous methods of experiment peculiar to itself and adapted to its own special purposes.

Medicine has at no time denied its dependence upon advances in the exact sciences, and so it is not strange to find that with *new* ideas in physics there have come corresponding periods of discovery in medicine. But the application of newly discovered facts in physics to medical problems for the solution of which they were never intended has as a rule brought it to pass that every era of progress has been followed by one of disappointment, a period characterized by an overgrowth of speculation and hypothesis.

The great development of mechanics in the seventeenth century associated with the names of STEVIN, GALILEI, KEPLER, DESCARTES, HUYGHENS, and many others fructified the epoch of the iatro-physicists whose accomplishments as evidenced by their work on the mechanics of joints and the development of Harvey's teaching of the circulation have lasted into modern times. But even

as late as the eighteenth century great *physicists* such as JOHANNES BERNOULLI attempted the solution of such subjects as *Dissertationes physico-mechanicae de motu musculorum et de effervescentia et fermentatione.*

In the first half of the nineteenth century the great development of physics, more especially electricity, favored the wonderful development of physical physiology which began its career in Germany.

But both these times physics were insufficient to exhaust the problem of life, and the fully developed reaction to the iatro-mechanical school finds counterpart in the reaction of modern times, the participants of which are divided between two camps. The belief of one of these, the neovitalists, can be traced back to the "anima" of GEORG ERNST STAHL. In this teaching vital force which has been so often pronounced dead is born again. The second group, not less dangerous than the first, employs an atomic mechanics for the explanation of life phenomena, and mistakes the death-dance of the molecules for living reality.

In this time of threatening retrogression the seeds of modern physical chemistry fall upon that narrow field of endeavor which we call our own. But if this new and flourishing science is not also to prove a hindrance to investigation by exceeding its natural limits, it is well that we define first of all the boundaries within which its laws hold in biological questions.

Let us attempt first of all to get a conception of the significance of the law of the conservation of energy as a means of biological research. This attempt seems all the more justified since OSTWALD, whose great services in the development of physical chemistry demand the

widest recognition, has already proclaimed the complete triumph of the *energetische Weltanschauung* (energetic conception of natural phenomena).

According to this conception transformations in energy constitute the kernel of all phenomena in nature, and their quantitative determination furnishes at the same time a complete insight into the course of things.

If this is true, then the reaction of the sensory nerves is also always a consequence of changes in energy and these become therefore the means by which sensory experience is obtained.

An attempt will be made in the following paragraphs to show that a purely energetic conception of natural phenomena conceals all the dangers of a too extensive generalization, as it leads to a one-sided development of our point of view with all its threatening consequences.

Do transformations in energy really constitute the whole or even the nucleus of the changes that go on, in and about us? Do we really react only in proportion to the amount of difference in energy ?

Transformations in energy are in fact constant accompaniments of all changes in nature, and we could scarcely possess a simpler picture of nature than one in which all differences represent only differences in the amount of of energy.　In reality, however, it is only one *side* of all natural phenomena that we are able to include in the energetic principle, for only for the *value of the mechanical work* performed in all changes does the law of its indestructibility hold.　The energetic analysis of a phenomenon is, however, so little exhaustive that in physical realms, such as that of electricity for example, we are unable to answer the question of the nature of electrical

phenomena in spite of most extensive utilization of the MAYER-JOULE law.

The energetic principle suffices equally little in biological questions, and we must regard the attempt of an excellent investigator to define general physiology as the energetics of life phenomena as not sufficiently comprehensive. Our law determines only the energy value corresponding with the changes that take place in living matter; the fundamental question of their nature remains entirely unanswered.

A picture of natural phenomena which shows only differences in energy is as incomplete as a photograph which shows only differences in light and shade.

Upon the second assertion of OSTWALD that we react only in proportion to differences in energy we must also place certain limitations.

What we designate as external stimuli are changes which also are connected with variations in energy. The important point in our question is whether differences in energy determine quantitatively the excitation value of a stimulus. If this is true, then electrical, thermal, or mechanical stimuli having the same energy value ought to possess the same excitation value. Things are by no means as simple as this, however. We do not perceive the energy communicated to our sense-organs directly. What we perceive are only changes in the state of our sensory nerves, a fact recognized by DESCARTES in his day and, as pointed out by JOHANNES MÜLLER, suggested even by PLATO.

When MÜLLER postulates in the famous laws which bear his name qualitatively different changes in state in each variety of sensory nerve, changes which for different

stimuli are of the same kind in the same nerve, this is not at all synonymous with saying: to equal amounts of energy equal reactions.

At different times and under different conditions we react differently to the same amount of energy, and conversely. Stimuli carrying an amount of energy which normally is not perceived can, as in strychnine poisoning, bring about most powerful effects. The selective behavior of the nervous end-organs must also be attributed to differences in the stimuli received, which are more than simple quantitative differences in energy. How great is the difference between our sensations of noise and of music! and yet the value of the transmitted energy in the two cases may be the same.

It would be an easy matter to increase the number of striking examples indefinitely. They all lead to the conclusion that the *quality* of a natural stimulus plays an important rôle, as well as the amount of its energy. MÜLLER himself is inclined to make a qualitative distinction between impulses when he speaks of homogeneous and heterogeneous stimulation of a sense-organ. After all that has been said the assertion seems justified that the new energetic world conception will prove to be scarcely less poor than the mechanical. Did we wish to go deeper we should have to call the former a purely *mechanical* one.

If with this we have to regard as a failure the attempt to solve from the standpoint of energetics DU BOIS-REYMOND's famous riddle of the universe, then of what value *are* the laws governing energy in the investigation of biological problems?

If we know from *experience* or if this leads us to assume

that two processes influence each other in the way, for example, that pressure affects the freezing-point of water or the electric current a magnet, the *degree* of this action upon each other can be directly deduced from the laws of energy.

If in the explanation of a phenomenon we build it up out of the elements $a, b, c, d \ldots$, then these elementary processes correspond with a group of transformations in energy which we will designate by $\alpha, \beta, \gamma, \delta \ldots$ The principle of energetics states that the sum of $\alpha + \beta + \gamma + \delta \ldots$ must be constant. The attempted analysis of the phenomenon into a, b, c, d is *possible only* when it satisfies at the same time, under the most varied circumstances, the above condition. The MAYER-JOULE law contains no more than this. But while it itself therefore gives no positive or complete insight into a phenomenon, it nevertheless renders possible the exclusion of a whole series of false interpretations of our observations. It constitutes, therefore, an indispensable and excellent control of our suppositions.

This control of our conceptions through the MAYER-JOULE law may be of two kinds. In the one case it will be able to prove that our assumption is wrong, in another that it is incomplete. In so far as it points out in the latter case an as yet undiscovered condition it seems under these circumstances to lead directly to the discovery of new facts.

But even under these conditions we learn from the law of energy only that something is to be sought or to be discovered. What this really is, or what the nature of the fact that is to be discovered is, can never be learned except through special investigation. We may say in

consequence that the energetic principle has really only a *formal* significance, and can tell us nothing regarding the quality of the process. This is apparent from its very nature, namely, that of combining equivalents. With what the numerical equivalence corresponds in any given case, of this the law tells us nothing; just as little as we know from the weight of an amount of gold, what kind of money it is, whether francs, marks, or guldens.

The law of the conservation of energy could not help but have from its very beginning an overwhelming effect upon every investigator, not only because of its great simplicity but also because of its unlimited tenability in all subjects.

It can seem little strange, therefore, that its heuristic value has often been overestimated. It is certainly going too far when, for example, a recognized medical historian says of the law, "The discovery of the law of the conservation of energy has contributed no mean amount toward disproving the vitalistic theory, that belief in a peculiar vital force, and toward proving that the laws of physics and chemistry suffice to explain all biological and pathological phenomena."

The darkness which envelops life phenomena cannot be illuminated through any principle of mechanics. For the time being, therefore, the belief in a vital force must needs continue to exist. As in the case of the other "forces" it too has had to bow to the law of energy, but its death-blow will be received only when our knowledge of natural phenomena will have attained a higher development than at present.

We have in the preceding paragraphs tried to go

back to the very foundations of modern chemistry in order to obtain a conception of its applicability to biological questions. In what follows an attempt will be made to ascertain the value of physico-chemical methods in special questions in medicine. A division of our subject might follow either one of two schemes. According to the first the division would follow that current in physical chemistry. A second, however, which seems better adapted to our purposes, takes into consideration the medical problems that have made use of the new methods.

We can readily distinguish between two fields of biochemical research. One in which dead material is studied and through a discovery of the structure of chemical substances, such as the proteins and carbohydrates, an explanation of their biological significance is sought; and a second which approaches the tissues and their functions directly and endeavors to unveil their secret vital activity through an investigation of their constituents and products. Often investigations that utilize more or less strictly the living organism have a knowledge of the results obtained in the first field upon which to base their work. True as seems to be the assertion that a broad chasm exists between the great group of proteins and living protoplasm, equally true is it that a bridge leads across this chasm, even though investigation has not yet succeeded in recognizing the nature of this connection. We may therefore expect that in the chemical and physical reactions of the proteins there exist even now many of the *elements* of physiological and pathological reactions. In fact this expectation has been fulfilled in great measure more especially

through a utilization of the methods of physical chemistry.

The studies of HOFMEISTER on the protein-precipitating power of salts have shown that these arrange themselves in the same order as they do when arranged according to their diuretic or cathartic action.

In a study of the condition of swelling which I published some time ago a large number of biological relations were found. Analogies exist, for example, between the absorption of water by substances capable of swelling and the absorption of water by the living organism; and the velocity of swelling and the time of a muscle contraction are about the same. A continuation of these experiments along the line of changes in the physical state of the proteins has led to results whose significance also extends beyond that for the dead material itself.

As is well known the proteins suffer when subjected to heat a change in state, a so-called coagulation, which, generally speaking, is not reversible. The coagulation point, that is the temperature at which this change takes place, is, among other things, dependent to a large extent upon the presence of neutral salts. If we employ the neutral salts in the form of equimolecular solutions, we can compare their effects on the coagulation-point, which may vary between more than fifteen degrees centigrade. If now we plot the concentrations upon the abscissas, the corresponding coagulation temperatures upon the ordinates, we obtain curves which give a general survey of the laws governing the process. From these we learn that with solutions of a medium concentration, the order in which the different salts follow each other when arranged according to their different acids is independent

of the base common to all of them. If the salts are arranged according to their bases, then they follow each other in a certain order which is independent of the acid common to all.

The effect of a salt upon the coagulation temperature is therefore made up of two components the effect of the acid and the effect of the basic parts. We call this an *additive ion effect.*

The remarkable phenomena observed when two salts S_1 and S_2 together affect the coagulation-point give us a deeper insight into the important biological relations existing between proteins and salts.

If in a protein-salt mixture to which a definite amount of S_2 has been added we allow the salt S_1 to vary in concentration, while in a second series of experiments we repeat this but use a different amount of S_2, etc., we obtain a group of curves. A second series of curves are obtained by using constant amounts of S_1 and varying amounts of S_2. In this way we obtain two groups of curves which illustrate very well the mutual effects of two salts upon protein coagulation. These curves show in a very remarkable way points at which they cross each other, in other words a constancy in the coagulation-point as soon as certain quantitative relations exist between the two salts. When this is attained a change in the concentration of one of the salts which at other times would bring about a change in the coagulation temperature remains entirely without effect even when the amount of the change is four or five times as great.

A point at which the curves cut each other may be shown also between the combination curve $S_1 + S_2$ and

the pure S_1 curve. In other words in a definite salt-protein mixture the addition of an indefinite amount of another salt does not change the coagulation-point.

These experiments, which are given here only in part, seem to show for the first time that compounds of a certain stability are formed when salts are mixed with proteins.

The additive ion effects of the salts show also a dependence upon certain quantitative relations. We must probably attribute this to an affinity between salt and protein which, as indicated by other observations, is of such a character that the metallic ion and the acid ion of a salt unite with different, asymmetric parts of the protein molecule.

An investigation of the conditions determining the solubility of egg globulin has shown that this is dependent upon the presence of ionized compounds. In even a highly concentrated solution of dextrose or urea, in other words substances which do not dissociate into ions, globulin is precipitated in the same way as in the almost entirely non-ionized water.

We may point out in passing the biological significance of these facts which show the importance of the mineral constitutents of the organism in a new light.

There can be little doubt that ion-protein compounds are present in the animal organism, in fact we have every reason for believing that all protein constituents of the protoplasm enter into the composition of this substance only in combination with ions.

As shown by numerous observations, the salts are held fast in the organism with great force. This affinity,

which until now could scarcely be explained, is an analogue of the affinity existing between salts and proteins as discussed above.

The way in which water and the way in which salt are united with the colloids have many things in common, for the water and salt mutually affect each other in the organism, and each of the two is maintained at as constant a value as possible. If we allow a swollen colloid to desiccate in an atmosphere which is kept permanently dry, we find that it loses water in a way analogous to that in which a protein-salt mixture loses salt when dialyzed against running water. In either case some water or some salt remains behind which can be removed only with the greatest difficulty if it can be removed at all. The ion-protein compounds show a considerable stability toward conditions influencing their state of aggregation in yet another way. The presence of neutral salts, for example, inhibits the effect of an acid or an alkali upon the dissolved globulin, while non-ionized substances do not do this.

We must therefore look upon the ion-protein compounds as being of importance in the animal body through their ability to decrease its sensitiveness toward changes in concentration, changes in temperature, and changes in alkalinity.

Through the decomposition of the large protein molecules and certain carbohydrates there are produced during metabolism substances having a low molecular weight which in many ways have characteristics in common with those of the salts,—such, for example, as urea or sugar. All these substances are either not at all or only slightly ionized in water. They are there-

fore not able to replace the salts which in dilute solution are almost completely ionized.

It is not the salts but the ions of the salts that are essential to the organism.

Our belief that many reactions of living matter can be traced back to the properties of the dead ground-substance itself seems to be true not alone of the proteins and their closely related bodies.

Emil Fischer has been able to show, for example, that the fact that enzymes or living organisms split certain kinds of *sugars* more easily than others is dependent upon their structural peculiarities, and has assumed the existence of a peculiar, stereochemical relation between the reacting substances.

A remarkable similarity between the reactions in dead and in living matter has also been proven to exist for the fats. If we allow two immiscible liquids, such as oil and water, to compete for a substance soluble in both, the amounts of this substance dissolved in the two solvents bear a definite proportion to each other. Meyer and his pupil Baum have studied a long series of narcotics with reference to their distribution coefficient in the above mixture, and have been able to point out a far-reaching parallelism between the distribution coefficient of a substance and its narcotic effect. This observation has led Meyer to an interesting theory of narcosis, based upon the difference in the distribution of the active substances between the watery tissue fluids and the fat-like constituents of nerve tissue.

We will now leave the field of more or less indirect biochemical investigation and, even though but hastily, consider the results which have been obtained through

a direct application of the new methods to changes which go on in the organism. These belong more or less in the field of special physiology, while the foregoing fall more naturally into the territory of general physiology.

We have to deal in what follows almost entirely with the principles of the modern theory of solution, of which extensive use is made in the explanation of phenomena of absorption and secretion.

We must consider it a great advance that we now know that almost all relations existing between the red blood-corpuscles and the plasma can be explained by the laws which govern any solution. The credit of having recognized this fact belongs chiefly to HAMBURGER and KOPPE. These investigations, it seems to me, are the first to conclusively do away with any higher life in the blood. What appears as life in the blood is only a reflection of those true vital processes which go on in the tissues. All known changes which take place in the circulating blood (with the exception of the white blood-corpuscles) are passive physico-chemical reactions, and are in consequence independent of nervous influence.

Of special physiological and pathological interest are the efforts to explain the activities of the kidneys from these new points of view. The starting-point of these is furnished by a paper of DRESER, in which this author develops for the first time the conception of the osmotic work of the kidneys and calculates this in mechanical work-units. A detailed study of this fundamental conception is desirable, since in its original form it is neither entirely clear nor complete.

We can determine the number of particles present in the unit volume of a solution, the so-called molecular

concentration, either through a determination of the osmotic pressure—the attraction between dissolved particles and solvents—or the change in the freezing-point or boiling-point of the solution.

All these values are related in a simple way and are independent of the nature of the dissolved substances. If we wish to increase the concentration of an aqueous solution we must remove a part of its water. It is immaterial whether we do this through evaporation, freezing, or expression of the water through a membrane impermeable to the dissolved substances, or whether this process takes place in the kidneys: in every case the amount of work required must, according to the laws of energy, be the same. The amount of this work is determined solely by the original concentration and the change in concentration finally attained. With these facts in mind, we will try to formulate the conception of *renal work* more clearly.

While the molecular concentration or the freezing-point of normal blood has an almost constant value, that of the urine varies within wide limits. Our kidneys are able to furnish a secretion the freezing-point of which may be higher or lower than that of the blood. For the sake of simplicity, it may be well to consider these two possibilities separately.

If the kidneys furnish a urine having a higher freezing-point than that of the blood, then their entire activity consists in the mere preparation of a dilute urine, and these organs do their osmotic work by expressing from the more highly concentrated blood a certain amount of water. During all this time the osmotic pressure of the blood is of course kept at its original height through the tissues.

The amount of water given off can be readily calculated: it is the amount which must be separated from the urine in order to change this into a liquid having the high molecular concentration of the blood.

The calculation of the *work* necessary to accomplish this is, however, not as simple as DRESER believes, since through the transport of the water from the blood to the urine the concentration of the latter is steadily altered, a fact which this author has not taken into consideration.

We will look upon the amount of work necessary for this purpose, which can be determined mathematically as a measure of the *water-secretory* function of the kidneys.

The converse of the above would exist when the kidney has to prepare, from a liquid having the osmotic pressure of the blood, one having a molecular concentration greater than the blood. Under these conditions we should have to add a certain amount of water to the urine in order to make its osmotic pressure equal to that of the blood. While in the first case, therefore, the kidneys have to express water from the blood, this time they have to express it from the urine and return it to the blood.

The work corresponding to this, which has been correctly determined by DRESER, we will have to regard as a measure of the *water-absorption* activity of the kidneys.

As we know from numerous facts, this double function of the kidney is performed by two different parts of the organ. While the glomeruli probably secrete the water of the urine, the uriniferous tubules have an antagonistic function.

The urine which we are able to examine has already been subjected to *both* kinds of work. We are able to

determine from it, by freezing-point determinations, only the *difference* between these two values, while the kidney has in reality performed the *sum* of the two. All the measurements of osmotic renal activity made by DRESER do not take this important fact into consideration. Failure to consider it must of necessity lead to radically incorrect conclusions.

The normal human being usually secretes a urine the freezing-point of which is lower than that of the blood, because its molecular concentration is greater. By consuming much water we are, however, able to raise the freezing-point of our urine, and it would not be difficult to so regulate by artificial means the amount of water taken up by the organism that the freezing-point of urine and blood would be the same. If now we base our calculation of renal work upon the difference between the freezing-point of the blood and that of the urine,—a difference which under these circumstances would be zero,—then we would be compelled to conclude that the secretion of a urine equimolecular with that of the blood had been accomplished without work, while as an actual matter of fact it may have demanded a great deal of work. For after what has been said it is clear that the osmotic work of water secretion and the osmotic work of water absorption by the kidneys equal each other in this case.

A statement which is found in various articles and which threatens to be adopted by text-books, that the work performed by the kidneys in twenty-four hours normally varies between 70 and 240 kilogrammeters, has therefore no real value. After what has been said it will not seem strange that the attempts to utilize for diagnostic pur-

poses the work of the kidneys as determined in this way have failed.

It seems to me that still another point should be noted. It is readily apparent when one studies the papers that have followed DRESER's initiative that the method of measuring the work of the kidneys as criticised above has been regarded as giving the value of the *total* work done. At the best, however, the method determines only that *portion* of the work which is necessary to bring about the secretion of the water of the urine. For our urine does not represent a concentrated or a diluted blood, but contains, as we know, the constituents of the blood in different concentrations, even when we disregard the osmotically inactive substances (albumin).

If we imagine a certain amount of urine having the same freezing-point as the blood, separated from this by a thin permeable membrane, and the blood kept in circulation and maintaining its original composition as in the body, an interchange between the diffusible constituents of the urine and those of the blood takes place until the amount of these is the same on both sides of the membrane. We will call such an interchange a molecular interchange, as does KORANYI, because the molecular concentration of the two fluids remains the same throughout the experiment. As we do not have to do in this case with differences in osmotic pressure, the *external* work is zero. But a certain amount of *internal* work is performed, the direction of which can also be determined. For substances migrate from regions having a higher concentration to those having a lower one, through which a certain amount of osmotic work becomes free for each of the substances. The *sum* of all these different

amounts of diffusion work would have to be used in order to separate the substances again and to bring about the original differences. This work must also be done by the kidneys, and in two directions, toward the urine and toward the blood. The value of this can also be calculated, a subject which is, however, not within the bounds of this paper. We may call this the *selective work* of the kidneys.

The osmotic work of the kidneys is therefore made up of three components—the osmotic work of water secretion, that of water absorption, and that of selection.

Dead material, such as gelatine plates, may also show a power of selection. For a recognition of this important fact we are indebted to HOFMEISTER and his pupil SPIRO. The latter has elucidated these phenomena through physico-chemical principles.

While, however, a gelatine plate that has absorbed a salt or a dye remains in equilibrium with its surroundings and is not capable of any further selective activity, the phenomena observed in the kidney are of a dynamic nature.

The selective function of the kidney is an uninterrupted process, maintained through the active metabolism of its living substance.

It is not possible to mention here all the other beautiful applications that have been made of physical chemistry to questions in physiology and pathology. Many important advances besides those already noted might be brought up here. Pharmacodynamics may also expect great changes through use of the new theories, as may be concluded from the attempts which are already being made to introduce these new methods into this science.

In fact, no branch of our science will attempt to solve the new questions presented to it without rich results.

I have arrived at the end of my paper, the purpose of which was to test the value of the methods of physical chemistry in questions of medicine. Unquestionably they enlarge that territory which the organic and the inorganic world have in common. The last barriers between the two cannot as yet be broken down, however, through the increase in our means of investigation that we are at present enjoying. There always remains an unsolved portion, the kernel, as it were, of vital phenomena.

The cause of the final failure of the new instruments can rest only in their origin. They have all been evolved from the study of *lifeless* matter. For a complete understanding of the living the words of a great physiologist will probably hold:

"*Life can perhaps be completely understood only through life itself.*"

2. The General Physical Chemistry of the Cells and Tissues.*

A COMPLETE and ordered understanding of all the functions of living matter, independent of its relation to a definite organism or organ, is the final goal of *general physiology*. Free from a one-sided overestimation of any one system of investigation, it makes use not only of the methods peculiar to biology, but also of those employed in physics and chemistry. The methods of chemistry have attained a special importance in the investigation

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* From Ergebnisse der Physiologie, 1902, I, 1te Abth., p. 1.

of the metabolic changes that take place in living matter. This so-called *vegetative physiology* has been greatly advanced through the modern development of theoretical or physical chemistry. It is the purpose of this young and rapidly growing branch of the inorganic sciences to establish the general laws governing chemical changes. Through the stupendous theoretical and experimental accomplishments of such investigators as VAN'T HOFF, ARRHENIUS, GIBBS, HITTORF, KOHLRAUSCH, OSTWALD, NERNST, and PLANCK, our understanding in this direction has within a comparatively short time been incredibly increased and deepened. As in every great development in the exact sciences, physiology may in this case also expect to be enriched in no small way, and though it to-day stands only at the beginning of this wonderful fertilization, the number of workers along special and general subjects in physiology is daily increasing. In fact, in the realm of general physiology the physico-chemical method of looking at things has been the first to make it possible to ask many questions in a general way and to answer them according to the present status of physico-chemical investigation. New analogies and transitions between phenomena in living and in dead matter have been discovered; and it has often proved no small task to discover that side of a phenomenon which characterizes it as a specifically biological one.

Important as many of the advances that have been made may seem, closer inspection shows that even at the best we are only beginning to solve the questions before us.

The development brought about through the seeds of physical chemistry has as yet not led to an equilibrium

between our imagination and fact, due in part to a lack of chemical data of biological importance, in part to a lack of that theoretical foundation necessary for special questions in biology. For this reason the following fragments of a general presentation of the physical chemistry of the cells and tissues cannot claim to be complete or to give a satisfactory account of facts to which nothing more will ever be added. It must suffice if the great importance of physical chemistry in general physiology is rendered apparent.

I.

All living matter is made up of colloidal and crystalloidal material, and there exists no life process that is not accompanied by changes in the colloidal and crystalloidal substances. And the physico-chemical laws which govern the crystalloids and the colloids reappear in the numerous properties of living matter.

The *colloids* have for the most part a high molecular weight, diffuse only with the greatest difficulty, and do not pass through animal membranes. Solutions of colloids have a scarcely measurable osmotic pressure, and have in consequence little effect in raising the boiling-point or depressing the freezing-point. They do not conduct the electric current, yet they move, for the most part, in an electric current.

The *crystalloids* diffuse easily and pass readily through animal membranes. Their molecular weight is low, while their affinity for water, as measured by an increase in the boiling-point or a depression of the freezing-point of their solutions, is very great. The crystalloids

are divisible into two groups—the *electrolytes*, which in (aqueous) solution conduct the electric current, and the *non-electrolytes*, which do not. To the first class belong the salts, acids, and bases; to the latter most of the organic substances, such as urea and sugar.

Between the two great groups of colloids and crystalloids there exists no sharp line, for we are acquainted with "half-colloids," which stand midway between these extremes. But because of the typical differences existing between the extremes of the whole series, differences which all substances show more or less perfectly, this division into colloids and crystalloids is nevertheless of great value.

The colloids exist in two states, a liquid and a solid state. In the liquid state they are known as *sols* (GRAHAM), in contrast to the solid state, in which they appear as dry, swollen, coagulated (through heat or ferments), or precipitated (for example, through electrolytes) masses, which are known as *gels*. A question that arises at once is, Do the colloids of living matter exist in the sol or in the gel state?

Protoplasm possesses properties which are characteristic, generally speaking, of both solid and liquid substances. This peculiarity of living matter has given rise to great discussions between the believers in the solid and those in the fluid state of aggregation of protoplasm. The ability to stand alone—in other words, a relative independence in form, which often expresses itself in the existence of characteristic cell forms—corresponds with the properties of the solid state, while an argument for the liquid state of protoplasm is readily found in the general and necessary condition that chemical reactions

must be able to take place within the cell and often with great velocity.

In those cases in which changes in the shape of the protoplasm under investigation can be easily explained through the assumption of the existence of a surface tension, there seems to be no reason for doubting the fluid nature of the protoplasm, for surface tension is ordinarily looked upon as a dependable criterion of the liquid state. The amœba, which becomes spherical in a state of rest or when universally excited, or forms pseudopodia when it suffers a local alteration in surface tension, may be looked upon as a liquid mass as long as it has not been possible to demonstrate in it a noticeable displacement elasticity such as torsion. To prove the existence of the latter by suitable experiment has never been attempted, so far as I know. Since the discovery of the "amœboid" movements of oil droplets and the careful physical analysis of this phenomenon by QUINCKE, the formation of pseudopodia has been robbed of the characteristics of a specific life phenomenon, and later investigators have shown that it is governed in all its details by the laws of surface tension. The taking up of food and the process of defecation in rhizopods can also be easily explained in this way. RHUMBLER could even imitate most cleverly with drops of chloroform and threads of shellac such apparently complicated phenomena as the rolling up of algæ threads within the body of *Amœba verrucosa*. By similar methods he was able to imitate in a most surprising way the formation of cases about testaceans by rubbing up fine quartz or glass powder with different kinds of oils or chloroform, and dusting this into dilute alcohol or water. E. ALBRECHT,

who has formulated the question of the state of aggre-
gation of living matter in both a penetrating and per-
tinent way and has attacked it with the armament of
modern physico-chemical research, could bring about a
separation of droplets within the contents of a number of
cells such as those of the sea-urchin and of the kidney.
Such a separation is dependent upon differences in sur-
face tension, such as can exist apparently only between
fluids. Even before him BERTHOLD had regarded the
normal formation of granules and vacuoles in protoplasm
as a separation of droplets. JENSEN has measured the
tensile strength of the pseudopodia of *orbitolites* and
has found that it about corresponds with the calculated
surface tension. This author has also again pushed into
the foreground the surface-tension properties of liquids
as a means of explaining many mechanical properties of
living matter.

In spite of these results, which are all of them, appar-
ently, capable of only one interpretation, a generalization
in the sense that *all* living substance must be liquid meets
with difficulties. The maintenance and the individuality
of form in cases in which no supporting framework is
demonstrable would have to be attributed by the believer
in the liquid state of protoplasm to currents that are
able to hold their own against disturbing forces. What
is of static origin in the solid state of aggregation needs
here a dynamic explanation which brings with it the
assumption of a constant expenditure of work. Since
an inner stabile differentiation is impossible in a liquid
(for even the finest particles of matter dissolved or sus-
pended in a liquid endeavor with great force to become
uniformly distributed throughout the whole), the assump-

tion of a liquid state of aggregation for protoplasm meets in many cases with still greater difficulties than does the assumption of a solid state. While, for example, all the different portions of the cell body of an amœba show the same behavior, in that any element within the protoplasmic mass may become a surface element, and conversely; in other words, every particle is equally capable of the functions of assimilation, stimulation, and movement, there exist peculiarities in many of the more highly developed unicellular organisms, or the individual cells of higher animals, which can scarcely be interpreted otherwise than as expressions of polarity. Under this heading belong, for example, the fact that absorption and secretion take place predominantly in certain directions, the dependence of muscular stimulation upon the angle of the current and the direction of the muscular fibrils, and the polarity of phenomena of regeneration in plants and animals. These phenomena, which indicate a persistent *inner* differentiation, can scarcely be explained without the assumption of a *solid orientation of the particles* of living matter.

A way out of this dilemma, of which the details constitute a literature that cannot be entered into in this paper, is rendered possible through a study of the *colloidal state.*

II.

Those gels which were said above to be swollen or solidified (for example, ordinary gelatine or agar-agar) show the properties of both solids and liquids united in one, in much the same way as protoplasm. They are capable of existing in all states of aggregation, varying

from the solid to the liquid, depending only upon the amount of water they have absorbed. Chemical reactions may take place anywhere in such a medium, and with almost the same velocity as in the fluid absorbed by the colloid alone. Such a jelly does not take up any other colloid which is brought in contact with it (differentiation between different membraneless cells), and a foreign colloid imbedded in it does not tend to spread (intracellular differentiation). Such gels undergo most delicately shaded changes in state even without changes in temperature, through the action of substances which are present in the living organism. They may be rendered more solid or more fluid, without suffering a change in the amount of water which they hold, through the action of crystalloids, and also through the action of certain enzymes (partial or complete peptonization).

Such solid colloids show yet other properties that have been used as potent arguments in favor of the entirely fluid character of living matter. If mercury globules are driven under pressure through a capillary into solidified gelatine, the gelatine closes in as completely behind the rapidly moving globules as a liquid itself. The separation of droplets such as ALBRECHT has described in protoplasm is also possible in solidified gelatine. In the fluid state, that is to say, above the solidification-point, gelatine is precipitated by certain electrolytes such as the sulphates, citrates, and tartrates of the alkali metals. As can be proved microscopically and mechanically, this precipitation is a separation of droplets—the appearance of a "phase" richer in gelatine. The precipitating power of the electrolytes decreases with an increase in the temperature, that is to say, a more con-

centrated salt solution is required to precipitate gelatine at a higher temperature than at a lower one. Corresponding with this, it is an easy matter to prepare concentrations in which a precipitation will not take place until a temperature below that at which gelatine becomes solid has been reached. When this temperature has been reached a precipitate is produced, this time also in the form of fine droplets, in the solid and originally entirely clear gelatine. We can therefore not regard such a separation of droplets as an undeniable proof of the fluid character of a medium. That, however, the application of the theory of surface tension to certain cellular phenomena may be of great service, as shown by the observations of ALBRECHT, is of course not questioned by the above experiment. Nor does anything stand in the way of looking upon the shrinkage forms of thin solidified gelatine (BUTSCHLI, PAULI) in alcohol, ammonium sulphate solution, etc., as expressions of surface tension. In fact, the similarity is very great between such shrinkage forms and the shapes of suspended ("schwerloser") masses of oil (cubes, cylinders, etc.) in suitable media, as described by PLATEAU. The difficulties that are encountered in endeavoring to explain, on the basis of the solid nature of protoplasm, the unhindered appearance and solution of crystals without the formation of holes, do not exist in the case of gels, as experiment has taught. LUDEKING has been able to demonstrate with the polarization microscope the appearance of ice crystals in the clear substance of thin slices of deeply cooled ($-18°$ C.) gelatine. One can also notice in salt-gelatines in which the crystalloid, such as ammonium chloride, shows a great fall in solubility

with a decrease in the temperature, that with a fall in the temperature supersaturation and finally crystallization of the salt occurs in the solid gelatine, and that when the gelatine is warmed once more the crystals disappear without leaving a trace of their existence behind them; and all this without a change in the state of aggregation of the colloid.

A study of gels has disclosed yet other interesting analogies with living matter. Variations in the degree of swelling or in the volume of jellies having the approximate size of body cells occur with a velocity the magnitude of which corresponds very well with that observed in the changes in volume noticed in living matter. Solid colloids also manifest very extensively a group of phenomena—so-called *adsorption* phenomena—the simplest laws of which still demand much study. In these phenomena a chief rôle is played by great surfaces which load themselves (depending upon the pressure, temperature, etc.), often very rapidly, with different substances. If one bears in mind the complicated combination existing in a solidified gel between the colloid and its absorbed liquid—the water seems to be held in part mechanically, in part in combinations varying from the most firm to the loosest, which renders possible true liquid and solid solutions in addition to pure adsorption—one is impressed with the great variety of ways in which substances may be taken up in such colloids. In this way a great *selection* in the substances offered them is rendered possible, as HOFMEISTER and SPIRO were able to illustrate with biologically instructive examples in gelatine and agar-agar plates.

Since a part of the imbibition fluid may be mixed

with ether-soluble substances—in the protoplasm these are such as cholesterin and lecithin, or, as OVERTON calls them all, *lipoids*—these gels are able to take up substances which are not soluble in water. The manner in which the lipoids are held by the cell plasma, the nature of which is still unknown, must no doubt be governed, according to our newer physico-chemical conceptions, by some property of the lipoids, such as their solution affinity. According to the extensive investigations of OVERTON, the ability of many substances soluble only with difficulty in water to enter the living cell is dependent upon their solubility in the lipoids.

If by the distribution coefficient of a substance between two solvents we understand the relation between the spacial concentrations which exist in these two solvents after equilibrium has been established, it is found that the distribution coefficient of many narcotics between oil and water determines also their distribution between medium (such as blood plasma) and cell contents (such as the lipoids of the brain), and therefore also their effect (MEYER, OVERTON). The investigations of FRIEDEN-THAL on absorption by the intestine of substances insoluble in water also belong under this heading, which is of such fundamental importance in many questions in physiology. SPIRO has given numerous examples of the general significance of the distribution law. No doubt experiments carried out on simple models would bring much light into this field.

After what has been said, the similarity in important physico-chemical properties between living matter and certain gels must be looked upon as an extensive one. Without doubt a continued investigation of the colloids

is destined to contribute much toward an understanding
of biological problems.

III.

The *sols* also play an eminent part in life processes.
In contrast to the green plants, which, according to well-
known cultural experiments, are able to obtain their
nourishment from pure crystalloidal solutions, animals
are dependent upon liquid colloidal food. The process
of digestion serves to prepare nutrient sols capable of
absorption, and fluid colloids are mechanically moved
about and distributed throughout the organism to the
nourishing tissue fluids. While exerting only a slight
osmotic pressure, the dissolved colloids are nevertheless
able, through their inability to pass through animal
membranes, to exert a resorptive power, which through
a steady change in the osmotically active material, as
maintained by the circulation, finally attains significant
proportions (OKERBLOM).

As recent investigations have shown, the sols have
several important properties in common with true sus-
pensions of very fine particles. The most important of
these from a biological standpoint is the at times enormous
surface effect of the colloidal particles contained in the
solution. BREDIG, who rediscovered the well-known
ferment-like action of metallic surfaces in the enormously
more active colloidal solutions of metals, and investigated
the whole subject quantitatively, has attempted to explain
the importance of the colloidal condition of the enzymes
by the enormous surface effects with which this condition
is combined.

The free surface energy and the osmotic energy seem

in many ways to bear a reciprocal relation to each other in the cell. The analysis of the colloidal material decreases the former while it increases the latter, and conversely. The metabolic changes which are forever going on in the living organ compel us to look upon life as a dynamic process, and the repeated attempts that have been made to comprehend life physico-chemically without taking this fact into consideration could not help but seem inadequate. To discover the right connection between this metabolic physiology and physical chemistry is among the most important of the problems of general vegetative physiology.

IV.

The biological significance of the *crystalloids* has until recently been the main object of research with the majority of those investigators who have made use of physico-chemical methods in physiological questions. Especially has use been made of the theory of solutions. The great fertility of VAN'T HOFF's teaching of osmotic pressure had as an immediate consequence its unrestricted application to all manner of life problems. The cells were looked upon as liquid masses surrounded by semi-permeable membranes which were supposed to act as PFEFFER's well-known model. A relative increase in the osmotic pressure of the fluids surrounding the cells was supposed to bring about a shrinkage, while an increase in the osmotic pressure of the cell contents over that of the surrounding fluid was supposed to be followed by a swelling of the cell. This so simple and consequently so enticing conception of the rôle of osmotic pressure in the organism meets, however, when further considered, with

difficulties; nor does it furnish even the possibilities of a complete understanding of many important phenomena. In spite of this, however, numerous fundamental investigations, such as those of HAMBURGER and KOPPE, continue to retain great value, representing as they do the first experiments undertaken in the study of a new subject. The principles employed in these investigations approximate actual conditions only more or less coarsely; they fail, however, to explain details because conditions for their employment in the organism are satisfied only *in part*. This fact leads, however, to the recognition of new physico-chemical peculiarities of living matter, as illustrated, for example, in such discoveries as the biological significance of the distribution law.

The osmotic relations between animal cells and their surrounding media were studied for the most part on red blood-corpuscles. These give off their red coloring-matter in dilute salt solutions as soon as the concentration of the salt drops below a certain value. The lowest concentration of different salt solutions which just prevent the "laking" of blood are said by HAMBURGER to be *isotonic* with each other and are regarded as bringing about the same degree of swelling in red blood-corpuscles. Since a determination of the osmotic pressures of such isotonic salt solutions by physical methods (determination of the freezing-point) showed them to be about the same, the "blood-corpuscle method" was looked upon as a universally applicable procedure for determining osmotic pressures. An extension of this method to a large number of crystalloids soon showed, however, that physical and physiological isotonicity are identical in only a few substances, the majority showing differences

between the two (HEDIN, GRYNS). A part of these exceptions could be explained by HEDIN, and more especially by KOPPE, on the ground that the crystalloids permeate the red blood-corpuscles more or less perfectly. KOPPE has taken the following stand: The solution of a red blood-corpuscle is analogous to the bursting of a balloon filled with gas in a rarefied atmosphere. This bursting will occur also when the space about the balloon is filled with a gas that can pass through the wall of the balloon, for it cannot under these circumstances counter-act the pressure existing within the balloon. In this way is explained the laking of blood in even the most concentrated solutions of substances which are able to pass into the red blood-corpuscles, such as urea.

If this simple conception, deduced from the analogy between gas pressure and osmotic pressure, is strictly tenable, then the addition of substances which lake red blood-corpuscles (such as urea) to salt solutions having a concentration in which the hæmoglobin just manages not to pass out of the corpuscles should be without effect, since the relative osmotic conditions within and without the cells remain unchanged. This is, however, not the case ; such solutions also become colored red. Whenever urea has been added to a NaCl solu-tion a much higher concentration of the latter is required to keep the red blood-corpuscles of the horse from losing their hæmoglobin than when pure NaCl is used. The differences in concentration varied in a series of experi-ments between 0.005 and 0.01 molecular NaCl, and were, strange to say, not markedly influenced by an increase in the concentration of the urea from 0.25 to 1.00 molecular.

Not until thorough investigations have been made

into the equilibrium between substances that dissolve and those that inhibit the solution of red blood-corpuscles, and the reversibility of the migration of color, can we know in how far hæmolysis through crystalloids represents a single process. The idea that the exit of hæmoglobin from the red blood-corpuscles does not represent a single change, as rendered apparent through the action of such various agents as electricity, cold, various poisons, etc., has found valuable support in the investigations of STEWART on the permeability of red blood-corpuscles to various salts (determination of electrical conductivity of plasma). Only the failure to recognize this fact is responsible for the extensive use that has been made of the determination of the osmotic resistance of the erythrocytes in the solution of questions in the physiology and pathology of the blood, for which this method was never adapted. We do not understand the conditions under which the physiological destruction of the red blood-corpuscles, with a splitting off of their coloring-matter, occurs, and neither the discovery of normal nor of pathological values for their osmotic resistance yields any data from which conclusions regarding their behavior under experimental (for example, removal of the spleen) or pathological conditions (icterus, hæmoglobinuria, etc.) may be drawn. In fact, the beautiful investigations of BORDET, EHRLICH, LANDSTEINER, etc., on the production of hæmolytic substances in the animal body point to a new field of work entirely outside of the teachings of osmotic pressure.

There is no objection to saying that several solutions which have the same osmotic pressure are isotonic, that is, isosmotic with each other; but to speak of the isoto-

nicity of a single solution is impossible, as KÖPPE has well pointed out by indicating the confusion wrought by this expression. The osmotic effect of a solution may be expressed in terms of *molecular* concentration (mol.). The molecular weight of a non-dissociable substance (such as cane-sugar), expressed in grams and dissolved in enough water to make a liter, constitutes the unit of molecular concentration. Such a solution has an osmotic pressure of 22.35 atmospheres at 0° C. Isotonic solutions are equimolecular.

The fact that there exists a difference between physical and physiological determinations of osmotic pressure is emphasized all too little. In the former case the pressure is measured against the solvent, in the second case the pressure of a solution against cells or their contents. Each of these values, differing as it does more or less from the other, may have its own biological significance.

A study of the changes in the volume of cells brought about through differences in osmotic pressure clearly shows that the conditions for the unlimited tenability of VAN'T HOFF's laws do not exist in the living organism. In its simplest form VAN'T HOFF's theory presupposes two things: impermeability of the separating membrane for the dissolved substance, and complete freedom of movement of the solvent throughout the entire medium contained within the membrane. If these two conditions existed in the case of cells, then two series of facts should be found to be true experimentally:

I. A cell should have the same volume in isosmotic solutions of different substances.

II. A cell should show an amount of change in volume

proportional to the amount of change in the osmotic pressure within or without the cell.

It was soon learned to attribute the exceptions to the first sentence to the relative permeability of the cells for certain substances. But this explanation does not suffice for a large and important number of cases in which the substance that has entered a cell exists here in a greater concentration than in the solution surrounding it. These cases have been explained in part through the distribution law.

Strange to say, not a single example investigated thus far has ever brought a confirmation of the second conclusion stated above—a change in volume proportional to the change in osmotic pressure. KOPPE, for example, found that it was the rule to discover very considerable variations from this law in the red blood-corpuscles. When the osmotic pressure of the surrounding liquid is increased, the decrease in the volume of the red blood-corpuscles is less than calculated, as is true also of the amount of their swelling when the osmotic pressure in the surrounding fluid is decreased. In the experiments carried out by DURIG on the swelling and shrinkage of frogs great exceptions to the simple laws of osmotic pressure were found to exist. We seem to have every reason for believing that freedom of movement and homogeneousness of solvent, which are demanded for an immediate application of VAN'T HOFF'S theory to the interchange between the fluids within and without the cell, do not exist in our tissues. A chief rôle in this modification of the solvent will no doubt fall to the part of the colloidal constituents of living matter.* The

* It does not seem impossible that the relations found to exist here

fact that protoplasm behaves in many ways as a mixture of different solvents might also be of importance.

V.

A peculiar and important place biologically is occupied by those crystalloids which because of their behavior in the electric current (conductivity and electrolysis) are called *electrolytes.* These are substances which in aqueous solutions (and in certain other solvents) break up into electrically charged particles, the ions (the electronegative anion and the electropositive cation). This electrolytic dissociation, which may in dilute solutions attain a very high grade, is, however, never complete; beside the ions there exist also non-dissociated, electrically neutral molecules. The investigation of the rôle of electrolytes in life phenomena must be directed toward an understanding of the part played by each of these.

Experiment has taught us that there exist physiological effects which are attributable solely to ions. The vital property of the ions to keep in solution the widely distributed globulins cannot be replaced by any other

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may be expressed mathematically. Support for this is found in the no longer negligible volume of molecules present in colloidal mixtures which bind the solvent. The water found in gels is, moreover, to be regarded as freely movable only in part, and this part decreases rapidly with an increase in the amount of shrinkage. In consequence of the increase in the values of the volume and the attraction of the gas molecules the simple equation $pv = R.T.$ no longer holds, as is well known, at higher temperatures, but VAN DER WAAL's equation $\left(p + \dfrac{a}{v^2}\right)(v - b) = R.T.$ Much seems to speak in favor of the idea that the relation between osmotic pressure and cell volume may be kindred to the latest modification of the law of Mariotte-Boyle.

kind of dissolved crystalloids (PAULI). The poisonous effects of water poor in ions upon the human organism (KÖPPE) may also belong under this heading.* Differences in the concentration of ions brought about through differences in their migration velocities constitute the source of differences in electrical potential. LOEB was probably the first who recognized in such "concentration chains" the cause of the majority of the electrical phenomena observed in animal organs.

We are far from a satisfactory insight into the nature of the effects of ions the elements of which may be electrical in character. Nevertheless, we know enough to be able to say definitely that a certain effect is quantitatively determined by ions, if it follows the general principles outlined below:

"Since very dilute solutions of electrolytes are almost completely dissociated, the effects brought about by such solutions may, as a rule, be looked upon as ion effects, and the electrically neutral molecules may be neglected because of their exceedingly small number. A pure ion effect must parallel the concentration of the ions and not the concentration of the substance itself; and at the same concentration of substance be dependent upon the degree of dissociation, which can be varied, without a change in volume, through the addition of an electrolyte having a common or a different ion. When anion and cation

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* A specific ion effect is not, strictly speaking, proved by this experiment, because the attempt to do away with the poisonous effects of the water through the addition of non-ionized substances such as sugar was not made. Moreover, NANSEN and his followers in his polar expedition drank for months without harm the almost ion-free water obtained by melting natural ice.

both take part in the ion effect it must be possible to demonstrate the law of additive ion effects. If, however, the ion effect is connected with but one of the ions, then it is dependent only upon the concentration of the effective ion, and a variation in the opposite ion must, under otherwise unchanged conditions, be indifferent. The rôle of the electrically neutral molecules springs into prominence in proportion as the degree of electrolytic dissociation is decreased."

Examples of such ion effects are to be found in the papers of DRESER on the pharmacology of mercury, of LOEB on the absorption of water by muscle and in his much-discussed experiments on artificial parthenogenesis, of HOBER on the sense of taste, of SPIRO with SCHEURLEN and BRUNS, as well as PAUL and KRONIG, on the foundations of disinfection, of PAULI on changes in state in the proteins, etc. The laws governing the simultaneous action of several electrolytes can also be deduced from the ionic theory. PAULI and RONA have recently discovered an antagonism between the effects of different electrolytes and some non-electrolytes on the changes in state in colloids.

In the living animal we have to deal with complex mixtures of crystalloids and colloids, between which there exist relations so varied that they are in part still incapable of investigation. Connected with the uninterrupted vital activity of the cell, the anabolism and catabolism of its substance, is the conversion of crystalloids into colloids and colloids into crystalloids, and this at present still entirely unexplained transformation serves at one time to protect a substance from oxidation, as in the conversion of sugar into the colloidal glycogen, while

at another it protects the protoplasm against the poisons
of its own products.   Questions in absorption, secretion,
pharmacology, and immunity are connected with these
changes in state, a discovery of the nature of which
must constitute one of the great aims of biochemical
research.

3. The Colloidal State and the Reactions that Go On in Living Matter.*

I.

COLLOIDAL material enters into the construction of
living matter in two forms—first, in the liquid or solid
state, in GRAHAM'S sense of the word, and, second, in
the form of a more or less solid, swollen mass, at
times sufficiently solid to have independent form, at
others, because of its approximation to the semi-solid
condition, still subject to the laws of surface tension
governing liquids.   We wish to consider in this paper
some of the properties of this swollen (jelly-like) condition
which can be attained not only through the absorption
of the so-called "solvent" by the original solid material—
for example, the absorption of water by dry gelatine—but
also through "gelation" of the liquid colloid.   This
gelatinous state is of the greatest interest to the biologist
in that it represents a state of aggregation in which the
properties of a solid and those of a liquid are often united,
a condition which is so frequently necessary in living
matter.   For this reason the experiments that have been

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* From Naturwissenschaftliche Rundschau, 1902, XVII, p. 312.
Address delivered before the Morphologisch-physiologische Gesellschaft
in Vienna, May 13, 1902.

undertaken to obtain a better knowledge of the inner structure of these jellies, partly from the standpoint of the morphologist, partly from that of the physical chemist, have not remained without influence upon important problems in general physiology.

The systematic investigations of BUTSCHLI, extending over more than a decade, tended to show that a fine honeycomb structure is present in all jellies, that, for example, ordinary solid gelatine when it has "set" consists of a framework made up of delicate gelatine walls, and that in this framework is contained a fluid gelatine of a low concentration. Expressed physico-chemically, every such jelly represents, therefore, a diphasic system, for we call every physically or chemically homogeneous constituent of a heterogeneous complexity a *phase*. Other investigators looked upon the results they obtained in expression experiments on jellies as furnishing further proof for the existence in them of a homogeneous fluid phase beside a solid supporting framework, and this in spite of variations in the amount of gelatine contained in the expressed liquid and its dependence upon the amount of pressure employed. These observations have led to the conclusion that living matter, too, is to be looked upon as made up of a honeycomb structure just as other colloids—a view which has been more and more adopted by physiologists and which has been utilized to render intelligible mechanical and chemical changes which go on in living matter. BUTSCHLI has, for example, used the radiating figures which appear about gas-bubbles in gelatine to explain the astrospheres which appear during cell division, for an undisputed similarity exists between the two pictures. Questions in absorption and the

spacial differentiation of chemical processes have also seemed capable of a seductively simple solution by belief in the existence of a finely chambered structure in living matter.

Against the very considerable evidence that has been brought forward for the existence of the honeycomb structure, any other conception of the constitution of jellies could hope to receive but little attention. Nevertheless, the attempt is once more to be made in the following pages to enter into a discussion of this difficult, but for the biological chemist so important, question of the structure of jellies. This will be followed by a discussion of the possibility of explaining certain fundamental properties of living matter which have been looked upon as an expression of its honeycomb structure independently of such a structure.

II.

Jellies are capable of a separation into two sharply defined phases; in other words, they can be precipitated or coagulated. Such a precipitation can be brought about, for example, through the addition of the sulphates, acetates, tartrates, or citrates of the alkali metals. For the sake of clearness we will base our considerations upon the behavior of gelatine, which represents one of the most thoroughly studied among the jellies. The separation during precipitation of a phase rich in gelatine from one that is poor can be observed not only microscopically, but also by allowing the precipitate to settle to the bottom of the vessel while kept in a thermostat, when the phase poor in gelatine forms a distinct layer

over that rich in gelatine. It will be well to compare first of all the laws governing this change in state, which is so well characterized through the formation of two phases, with the laws governing the solidification or gelation of colloids, for which, as has already been discussed, a separation into two phases is also looked upon as a distinguishing characteristic.

Investigations which have been carried on during past years have, however, disclosed a whole series of marked differences between these two processes, the more important of which are now to be briefly touched upon.

The gelation velocity and the gelation-point of gelatine are always more or less influenced through the addition of crystalloids, which at times hasten, at other times inhibit gelation, when compared with the gelation when pure water only is used. This influence of the crystalloids is a progressive one and increases in proportion to the amount added.

The precipitation of gelatine is, on the other hand, strictly connected with the addition of *definite* amounts of the precipitating agent, amounts less than are sufficient for actual precipitation being without effect.

While *all* crystalloids modify the process of gelation, even though they do this in different degrees and in different directions, only *certain* crystalloids are precipitating agents, while the others do not possess this power even in the most concentrated solutions. Gelatine, for example, is precipitated only through certain electrolytes, non-ionized crystalloids being effective at no concentration. Non-conductors, such as urea and dextrose, can, however, influence the process of gelation just as effectively

as electrolytes, and this in both directions. Urea inhibits gelation, while dextrose favors it.

If gelation consisted fundamentally in the formation of a phase rich in gelatine, then one would expect to find all precipitating salts among those substances which favor gelation. This is true, however, of only a part of the precipitating agents; the precipitating chlorides of potassium and sodium exert even a liquefying action upon gelatine to a considerable extent.

The following fact also speaks in favor of a strict separation between the two kinds of changes in the colloidal state.

If the gelation-points are plotted as ordinates, the molecular concentrations of the added crystalloids as abscissas, one obtains curves which readily indicate the dependence of the gelation upon the added crystalloid. These curves show no irregularities throughout their course, not even when the amounts added approximate very closely those at which precipitation occurs.

When several substances are allowed to act together, the differences between the laws governing precipitation and those governing gelation also evidence themselves. It could be shown on a large series of crystalloids that when more than one are allowed to act simultaneously upon a colloid the effects of the separate crystalloids upon *gelation* add themselves algebraically. This summation of the effects of the individual crystalloids is not altered when electrolytes are combined with non-electrolytes, or these with each other, nor by the fact that through the combination of electrolytes having a common ion the degree of dissociation is reduced, nor by the valency of the ions.

But matters are entirely different when the gelatine

is *precipitated.* The meeting of two electrolytes with a common ion favors precipitation, while certain non-electrolytes, such as urea and sugar, inhibit through their presence the precipitating power of electrolytes, and even cause already existing precipitates to go back into solution. These differences between the two changes in state may be made still clearer by citing a few examples. Thus gelation is greatly inhibited through the presence of bromides, while precipitation through electrolytes is markedly increased as soon as the added bromide contains a common ion. The combination sodium acetate—sodium bromide with the common Na ion is in this way a more powerful precipitating agent than the acetate itself. Again, dextrose favors gelation in that it elevates the gelation-point and increases the gelation velocity, and yet it inhibits the formation of a precipitate through a precipitating electrolyte as soon as the sugar is present in sufficient amount.

The independence of the two changes in state evidences itself in this also, that through suitable selection of experimental conditions it is possible to produce precipitates in a solid and clear gelatine just as in a liquid one, and this without a change in the state of aggregation of the solid gelatine.

It seems to me that what has been said proves without question that between the precipitation and the gelation of a colloid, at the basis of which fundamentally similar changes have been supposed to lie, there exist in reality profound differences through which the assumption of a related origin of the two phenomena has been rendered most improbable. In the same direction will be found to point an analysis of those facts which have always

been considered as sufficient evidence for the primary coagulation structure of all solid colloids.

III.

The beginning of those investigations of BÜTSCHLI on colloidal structures which must be considered in the general question that lies before us were observations on microscopic foams of gelatine and olive-oil, which when properly prepared furnish a framework of solidified gelatine walls in the chambers of which is inclosed the fluid oil.

BÜTSCHLI found that the structures which can be obtained through typical coagulation of colloids are also built according to this plan. Thin layers of egg albumin coagulated through heat or those precipitating agents which are generally known as "fixing-agents," or acacia solutions precipitated with alcohol, precipitated liquid (peptonized) gelatines, etc., all show under the microscope the same characteristic, finely honeycomb structure. Up to this point the conclusions of the Heidelberg zoölogist, which are of great interest to the molecular physicist also, show a complete harmony between observation and interpretation; and the value of BÜTSCHLI's discoveries for the morphologist who utilizes analogous methods to render apparent cell structures is not to be underestimated.

In the further course of his observations on colloids BÜTSCHLI later concludes, from a study of substances in the condition of swelling (jellies), that these also have a true honeycomb structure identical with that observed in coagulation foams and in typical coagulations. Only

this honeycomb structure is ordinarily not convincingly demonstrable; it becomes distinctly visible, however, under certain experimental conditions.

A closer study of the conditions which make apparent the honeycomb structure teaches us, however, that we have to deal in every case with the introduction of true coagulation or precipitation phenomena governed by the laws already outlined above. By far the most thorough investigations bearing upon this subject have also been carried out on solidified gelatine, and upon these BUTSCHLI supports in the main his belief in the primary honeycomb structure of all swollen media, and consequently also that of native protoplasm.

But, as has already been pointed out, even though neither direct observation nor tinctorial methods—and this in spite of the well-known marked affinity of gelatine for dyes—have rendered it possible to prove the existence of a honeycomb structure in untreated gelatines, such a structure is, nevertheless, supposed to exist in all probability. The reasons which BUTSCHLI has brought forward in support of this idea can only very briefly be given here and their tenability be tested.

The reason why it is normally impossible to see the walls constituting the framework of gelatine is, according to one author, dependent in part upon the fact that they are pliable and when dried in vacuo, for example, adhere closely to each other, in part upon the fact that the difference between the indices of refraction of the walls of the framework and of the substance found within them is too little to give distinct pictures. These walls of the honeycomb structure can, however, be rendered more solid and dense through the action upon them of

chromic acid, alcohol, or ether, when their optical recognition is made much easier. Any one can at any time prove to himself that a thin layer of gelatine when treated with dilute chromic acid according to BUTSCHLI's instructions becomes opaque and white, and shows under the microscope a very regular, finely chambered structure entirely identical with the picture found in true coagulations of colloids. But we are supposed to deal here not with a true coagulation, but, as BUTSCHLI expresses it in a by no means clear and unequivocal way, with a "kind of coagulation" through which a preformed structure becomes visible.

That structures which have been produced through the action of alcohol disappear when put into water, to reappear in exactly the same form when subjected a second time to the action of alcohol, does not argue at all in favor of the primary nature of the structure. The explanation of this phenomenon is easily found in the well-known properties of the changes in state which colloids suffer. The change which is brought about in gelatine through alcohol is, in contrast to that produced through chromic acid for example, simply reversible. Since, however, changes in state take place only very slowly in gels, these are, if at all, only gradually and scarcely completely reversible. It is readily intelligible, therefore, why under these circumstances structures which have once been produced and which are not entirely destroyed even through remelting of the gelatine reappear in their old form.

That a fluid condition of the colloid is necessary for the production of coagulation structures, as BUTSCHLI believes, and that these structures must be present

as soon as the colloid solidifies, can be readily shown to be untrue through the production of precipitates in clear, solidified gelatine. For, since the precipitation limits of many electrolytes, such as the sulphates, citrates, and tartrates of the alkali metals, are dependent upon temperature in such a way that they are lowered with a decrease in the temperature, it is an easy matter to prepare salt-gelatines in which heavy precipitates do not appear until the solidification temperature has been long passed —in other words, in the clear and solid gelatine. Such coagulations are subject to the well-defined effects of coagulation "germs" in the same way as those which occur in a fluid medium. The beautiful figures which LIESEGANG has been able to produce in colloids with the aid of precipitates all rest, in the main, upon the "germ" action of previously produced coagulations. In such processes is also found an unforced explanation of the phenomenon, used by BÜTSCHLI as an argument in favor of the existence of a primary honeycomb structure, that delicate granules when suspended in gelatine are always found in the nodal points and walls of the framework. The reason for this is that these foreign bodies all act as coagulation germs. For the same reason, the chambers of the honeycomb structure arrange themselves in rows which correspond with the lines produced on the slide in polishing it. And just as little will we be able to consider it proof of a preformed foam structure that the coagulations which take place in colloids mirror all the stresses that appear in it, due in part to shrinkage while drying, in part to the contraction of cooling air bubbles.

As further support for belief in a preformed structure,

observations are utilized which are noticed when delicate
gelatine threads that have been kept in absolute alcohol
or have been dried in the air are subjected to the effects
of tension or pressure.   If such threads are stretched or
bent, microscopic examination reveals a cross-striation
upon their surface corresponding with parts of the
threads that have become white and opaque.   The cen-
tral portion of the threads may retain its hyaline char-
acter.   That we are dealing in this case with more or
less well-marked breaks in continuity is without question
when the manner of their production is considered.
BÜTSCHLI explains the regularity of the pictures which
are produced by saying that the chambers of the stretched
honeycomb structure of the gelatine give rise to a system
of stripes which cross each other diagonally, just as is
the case with a net when this is pulled in certain direc-
tions.   It would be an argument in favor of this explana-
tion if it could be shown that in gelatine threads which
had previously not been treated with fixing-agents the
distance between the stripes is less than the diameter of
one of the honeycomb chambers.   This is, however, not
the case.   In a large number of measurements BÜTSCHLI
has determined the diameter of the latter to be 0.7 μ in
gelatine that has been treated with chromic acid or
alcohol, while the distance between the stripes in untreated
gelatine threads is 2.1 to 2.3 μ.   That the honeycomb
structure of gelatine threads which have been treated
with precipitating agents is more or less cross-striated
cannot seem strange when the systems of cross-striation
are looked upon as expressions of a definite distribution
of tension and pressure in the threads.   As has already
been described above, such stresses may impress them-

selves upon coagulations also, and under favorable conditions may evidence themselves even about fine suspended granules. A satisfactory explanation of the fact that tension alone may make a honeycomb-like structure visible, BUTSCHLI is unable to give, because of lack of observations directed toward this point. But surface structures similar to those described above frequently appear in different substances that have been stretched or compressed, in part as an expression of the incomplete mechanical homogeneousness of these bodies. Honeycomb and fibrillar pictures are found on the surface of stretched and compressed metals, and we can justly put into this class also the cross-striated structures observed by BÜTSCHLI on delicate threads of Canada balsam, without assuming, with this author, that this resin also possesses a preformed honeycomb structure. Interesting and worthy of further study as these observations may be, they furnish conclusive evidence of the primary honeycomb structure of colloids just as little as the already described experiments dealing with the question of rendering this structure visible. That we are dealing with a true coagulation whenever a structure is rendered visible, and not, as BÜTSCHLI thinks, with a condensation of primary supporting walls, in a certain sense a more advanced stage of simple gelation, is shown by the following experiment, which to my mind is conclusive.

In order to recognize its nature, let us recall to mind the already discussed laws governing coagulation, on the one hand, and gelation, on the other, under the influence of combinations of electrolytes and non-electrolytes. These two classes of substances add themselves algebraically in their effect upon gelation, while the coacer-

lation brought about through electrolytes does not occur if such non-electrolytes as urea or sugar are present; in fact, an already existing coagulum is made to go back into solution when these substances are subsequently added. If thin layers of gelatine spread upon slides are introduced for fifteen minutes into a 0.3 per cent. chromic acid solution kept at an even temperature of about 23° C., the beautiful coagulation structures are produced which BÜTSCHLI has described and pictured. As soon, however, as urea is added to the chromic acid solution in the concentration of 1.0 molecular, the gelatine does not become opaque, and a formation of structure as described above does not take place, even when everything else in the experiment is arranged as before. The gelatine remains clear, and examination with even the highest powers of the microscope shows it to be homogeneous. Urea in the concentration of 0.25 molecular is without effect, while concentrations above 2.0 molecular lead to excessive swelling and solution of the layer of colloidal material. That we are dealing in this experiment not with the inhibiting effects of urea upon gelation, but with its anti-coagulating effects, is shown by the following. Chlorides cause an excessive swelling and prevent gelation in the same way as urea, a 2.0 molecular sodium chloride solution* being equal to a 1.0 molecular urea solution. Yet chlorides have as electrolytes no inhibiting effect upon coagulation. Corresponding with these facts, it is found that an addition of sodium chloride to the chromic acid solution equivalent in effect to an adequate amount of urea does not at all prevent the formation of the typical coagulation structures in the gelatine preparations. These experiments prove definitely that in the

formation of structures in gels we are dealing with true coagulations. The experiments may easily be varied and similar results be obtained by using other fixing agents and non-electrolytes. It may be pointed out, in passing, that histology could easily employ to advantage this property of the non-electrolytes, especially that of urea, for obtaining a finer gradation in its methods of hardening and fixing tissues, as well as for causing changes brought about through these methods to disappear more or less perfectly.

All physico-chemical investigations that have been described here indicate, therefore, *that the condition of swelling in colloids is not to be looked upon as a diphasic one,* and that the reasons which have thus far been advanced in favor of such an assumption do not bear careful criticism. We can therefore find in the properties of the jellies no arguments for believing that protoplasm is a strictly diphasic system having a finely honeycomb structure. No doubt the substance of the cell may, in those instances in which we have to deal with inclusions of such substances as colloidal carbohydrates or fats, represent a heterogeneous complexity with phases the relations of which to each other are subject to the laws of chemical equilibrium. In general, however, such inclusions take part only indirectly in the actual life processes of the cell. At present no cogent reason exists for not believing that the mass which is looked upon as the bearer of life processes may not well be monophasic in structure.

According to a view expressed years ago and based on studies of the way in which water is held in gels, the colloidal particles of the latter are believed to contain

the liquid producing the swelling in combinations vary-
ing from those that are exceedingly loose to those that
are very firm. In this way is rendered possible a great
diversity in absorption phenomena as well as the formation
of solid and liquid solutions. From this metastabile con-
dition of equilibrium the gel gradually endeavors to
attain one in which all the colloidal particles are swollen
to the same degree. In living matter colloidal material
is being constantly broken down and built up anew, and
in this way the progress toward a final condition of
equilibrium in the molecular disposition of the liquid
producing the swelling is steadily destroyed.

IV.

The colloidal constitution of living matter is intimately
connected with one of the most important problems in
biological chemistry, i.e., with the question of the spacial
differentiation of the chemical reactions in protoplasm.
Since colloids resist the diffusion into them of other col-
loids, it is self-evident that through the presence of
different colloids within a cell as many different localities
are provided in which chemical reactions having a more
or less different course may take place.*   With the
exception of these coarser divisions between chemical
reactions, physiological experience compels us to believe
that chemical reactions of the most different kinds are
simultaneously possible in the homogeneous, colloidal
ground-substance of the cell. In even the smallest par-

* The great importance of the differentiation of the cell into nucleus
and cell body has been proven beyond question. Reactions that are
connected with the heterogeneous constitution of the cell no longer
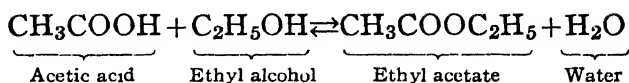take place when the cell is destroyed mechanically.

ticles of protoplasm antagonistic chemical reactions, such as oxidation and reduction, hydration and loss of water, condensation, polymerization, synthesis, and their opposites, or, generally speaking, as HERING puts it, assimilation and dissimilation, are able to occur through and beside each other.

In a suggestive lecture on the chemical organization of the cell, one of the greatest of present-day biochemical investigators has thrown much light on this important problem and has assumed for its solution the existence of a finely chambered structure in colloids and the impermeability of the colloidal walls.* Just as the chemist allows different chemical reactions to take place in different vessels, the cell is believed to utilize the different chambers of its honeycomb structure and, with the help of the colloidal ferments, the number and knowledge of which is daily growing, allow the necessary reactions to go on independently of each other.

As the considerations outlined above have shown that we lack at present any adequate foundation for believing that living matter has a honeycomb structure, the question arises whether it is possible for antagonistic chemical reactions to take place in exceedingly small spaces without the help of any structure. An analysis of such antagonistic reactions brings with it, I believe, their satisfactory explanation, based upon numerous facts and teachings of physical chemistry.

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* HOFMEISTER (Naturw. Rundschau, 1901, XVI, p.581) supports the hypothesis of a finely chambered structure in protoplasm upon chemical grounds which need not be discussed here. We have first to settle the question whether antagonistic reactions can at all take place in a homogeneous substrate.

The chemical reaction

$$CH_3COOH + C_2H_5OH \rightleftharpoons CH_3COOC_2H_5 + H_2O$$

<u>Acetic acid</u>   <u>Ethyl alcohol</u>   <u>Ethyl acetate</u>   <u>Water</u>

furnishes a picture of a simple reaction which may take place in either direction. If we begin with a mixture of chemically equivalent amounts of acetic acid and ethyl alcohol, the reaction takes place from left to right, with the formation of ethyl acetate and water; and conversely, if the latter are added to each other, the reaction takes place in the reverse direction. Whatever the starting-point, the final result is a definite state of equilibrium between all the end products. Depending upon the direction toward which the equilibrium-point is pushed, at one time the one reaction, at another time the other, may have the upper hand. The manner in which such an equilibrium-point can be displaced is illustrated by the change that systems such as the above suffer under the influence of an increase in temperature. According to a well-known law, an increase in temperature pushes the equilibrium toward the side of the endothermic reaction.

The following equations, in which letters have been used in place of different chemical formulas, illustrate some types of such reactions which in practice take place only in the one or in the opposite direction:

$$\left.\begin{array}{l} A + m = B \\ B - m = A \end{array}\right\} \text{I.}$$

$$\left.\begin{array}{l} (\alpha + \beta + \gamma + \ldots) - m = M \\ M + m = \alpha + \beta + \gamma + \ldots \end{array}\right\} \text{II.}$$

One can at any time imagine either O or H_2O written into equation I instead of *m*, and so obtain the picture of a simple reversible reaction—an oxidation and a reduction, or a hydration and a loss of water, following the type of a simple reaction which may take place in either direction; or in equation II a condensation or a hydrolytic cleavage. General biochemistry has until now taken notice of only this type of antagonistic reaction, that is to say, reactions which counteract each other in the sense of positive and negative values.

There exists, however, among the reaction chains in the body a second apparently very common type of antagonistic reaction, the nature of which can be best illustrated by certain changes in physical state that colloids are capable of suffering.

It is a well-known fact that with the customary methods of investigation it is found that the melting-point and the solidification-point of the crystalloids coincide. It is different, however, in the case of the colloids, in which these two points may lie some distance apart, even when the changes in temperature are brought about most carefully. In consequence of the indolence with which changes take place in colloids, superheating and undercooling are the rule. A gelatine the temperature of which lies between the melting-point and the gelation-point shows in consequence a peculiar behavior. If such a gelatine is cooled to beyond the gelation-point and is then carefully warmed back to the original temperature, the gelatine remains solid; if, however, the gelatine is heated to beyond the melting-point and is then carefully cooled down to the starting temperature, it remains liquid. A change in state, therefore, impresses itself upon

the colloid and determines the condition in which the colloid will ultimately be found.   In other words, a colloid seems to remember more or less perfectly a change that it has suffered, just as does living matter.   If gelation and melting followed the same course, only in opposite directions, then a gelatine, when it has returned to its original temperature, should also be existing in its original state, no matter in which direction it had previously suffered a change in temperature.   That we have to do in this case with reversible changes that follow *different paths* is evidenced by another property of this change in state.   If one compares the curves indicating the melting- and gelation-points of gelatines of different concentrations, obtained by plotting the concentrations upon the abscissas and the corresponding melting- and gelation-points upon the ordinates, it is seen that the two processes are dependent in different ways upon the concentration of the gelatine.   The gelation curves follow an approximately straight line; the melting curves, on the other hand, rise gradually, but in a decreasing degree, above the abscissa.

In contrast to the previously described *simple* or *homodrome* antagonistic reactions, which follow the same course in either direction and which behave at any stage as mathematical values having different signs, we are dealing in this case with *complex* or *heterodrome* antagonistic reactions, which reach their respective end states along different paths.   (See Figs. 1 and 2.)

In what follows we will make use of these simple diagrams in characterizing the antagonistic reactions.

Heterodrome reactions of a chemical nature play an important rôle in the changes that go on in living

matter. They may be readily illustrated by a few examples.

The equations

$$\left. \begin{array}{l} A + m = B \\ B - m' = A' \end{array} \right\} \text{ I}a$$

$$\left. \begin{array}{l} a + b + c + \ldots = A \\ A = l + m + n + \ldots \end{array} \right\} \text{ II}a.$$

represent, when m and m' indicate differently placed O or H_2O groups, cases in which, as in Ia, oxidation
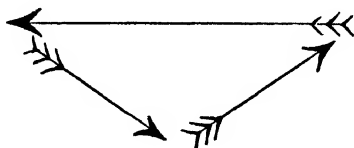

FIG. 1.   FIG. 2.

and reduction, or hydration and splitting off of water, follow a heterodrome course; or, as in IIa, cases in which the decomposition products are different from the substances employed in the synthesis.

The principle of antagonistic reactions remains the same whether they take place under the influence of substances that increase the velocity of the reactions, so-called catalyzers,* or not. In the organism we have acting as such catalytic agents mostly ferments, which are capable of acting only upon certain substances, and

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* No doubt the catalyzers determine also the *direction* of a reaction, in that the reaction follows a qualitatively different course under the influence of different catalytic substances. If one holds fast to the above-mentioned (OSTWALD'S) definition of catalysis, then we would be dealing in this case with several simultaneously possible reactions, of which different ones are accelerated by different catalyzers, while the rest remain not discoverable.

playing a rôle, therefore, only when these substances appear in the organism.

Such catalyzers can act, as was first pointed out by VAN'T HOFF, in two directions, depending upon the relations existing between the substances originally present and those formed. In this way, as has recently been shown, amygdalin cannot only be split into amygdalic nitrilglucoside and glucose under the influence of yeast maltase, but also be formed synthetically from these two substances with the help of the same enzyme. One and the same enzyme can, according to conditions, accelerate the one or the other homodrome antagonistic reaction. In the animal organism the synthesis of glycogen from dextrose and the splitting of glycogen into dextrose might in part, at least, represent a simple antagonistic reaction governed by a single enzyme; while the synthesis of starch in plants and its diastatic splitting into glucose or maltose represents a heterodrome antagonistic reaction in which the synthesis has the upper hand by day and the analysis by night.

It is also possible, however, that *two* catalyzers may act in such a way upon a homodrome antagonistic reaction that the one accelerates the conversion of the system in the one direction, while the other does it in the opposite direction. This is the case when the two catalyses go hand in hand with different equilibrium-points in the two reactions that constitute the simple antagonistic reaction. An example of this sort will be given later. The deposition and solution of the calcium salts of the bone represents a simple reversible reaction which, with the aid of special cells, the osteoblasts and osteoclasts, can, according to physiological needs, be divided, and

the two reactions occur independently of each other in entirely separate localities.

In heterodrome antagonistic reactions the acceleration of the two reactions by one and the same catalyzer is impossible; one catalyzer can be effective in only one of the two reactions. A reaction can take place in one direction, and at a certain stage—for example, after a condensation or polymerization through one enzyme—be switched into another direction; or the components of the complex antagonistic reaction may be influenced through two different catalyzers. Between two catalyzers which displace the equilibrium of a homodrome antagonistic reaction in opposite directions, there exists a true antagonism, while a complex antagonistic effect exists where the two components of a heterodrome reversible reaction are governed by two catalyzers. We may therefore distinguish also between *simple* and *complex* antagonistic ferments.

A suitable example of a heterodrome antagonistic reaction in metabolism is furnished by the formation and destruction of uric acid in the animal body. The fundamental investigations of H. WIENER have thrown light upon this subject. WIENER found that the " surviving" ground-up pulp of different animal organs has the power of both forming and destroying uric acid. In the liver of the ox the two processes can take place simultaneously and are, no doubt, dependent upon the activities of two different catalyzers. As the sensitiveness of the two catalytically acting substances toward heat is different, the two chemical changes can be separated from each other, the power to decompose uric acid being lost later than the power to form it. That we have to do here

with a heterodrome reaction is indicated by the fact that none of the substances which are formed in the destruction of the uric acid can be built up into the original uric acid.

It would lead us too far afield to discuss further the fate of the different substances that enter into the metabolism of the organism in the light of our conception of antagonistic reactions which can be combined among themselves in the greatest variety of ways. In the last analysis, no doubt, it is because the reaction is a heterodrome one that no path exists in the animal body over which urea can again be built up into protein.

The two components of an antagonistic reaction are dependent upon each other only in so far as the one furnishes the material necessary for the other. We can easily see how in the fact that they can follow different courses there resides the possibility that they can take place simultaneously and side by side. This explains also why ferments acting in opposite directions can exhibit their characteristic effects without the presence of separating walls—in molecular proximity to each other, as it were.

Such reactions can without mutual interference take place side by side, just as sound, light, and electric waves, or currents of heat, electricity, and diffusion, can pass through a medium simultaneously.

V.

The idea of homodrome and heterodrome antagonistic reactions, as deduced from a consideration of changes in the colloidal state and in metabolism, is closely related

to facts which furnish a welcome support of this conception in the physiology of the senses.

As early as 1865, through his complete recognition of the relation between the physical and psychic elements of a sensation and through the assumption that every quality of a sensation has lying at the bottom of it a specific change in the substance of the nerve, which for the same sensation is the same, and for similar sensations partly identical. E. MACH.laid the foundations from which, through an analysis of the sensations, a knowledge of the changes that go on in living matter has been obtained. In his hands and through the work of E. HERING, who, somewhat later, and independently of MACH, set up the same principle of research, this led to a great enrichment of the physiology of the senses. Upon these same foundations HERING has built up his famous theories of the sense of light and color, a theory of the temperature sense, and finally a general theory of the changes that go on in living matter. The great and fruitful significance that these principles possess, not only for questions in the physiology of the senses, but also for general physiology and for a recognition of the aims and limits of scientific research in general, could only temporarily be belittled, through the mighty authority of a HELMHOLTZ. To-day when this combat is a thing of the past and the teaching of MACH and HERING has made itself felt in the most varied branches of science, we recognize that in decades general physiology has enjoyed no such significant increase in enlightenment as has been furnished by the MACH-HERING analysis of the sensations and the physical changes that lie at the bottom of these sensations.

We will enter into this physiology of the senses only

far enough to show that it contains all those elements which we discovered in the discussion of another subject. The idea that antagonistic reactions must be possible in even the smallest particles of protoplasm we meet in HERING's theory of the changes that go on in living matter. In the discussion of assimilation and dissimilation, he writes:

" But in separating the mental conceptions of these two processes we must not be misled into thinking of them as two processes which, while they go on side by side, are really separated, and into imagining living sub-stance to be within itself a resting mass that on one side only analyzes matter and on the other only synthesizes it; but rather as a copper wire dipping with both its ends into copper sulphate, which when it is traversed by an electric current suffers at one end a loss of copper by going into solution, while at the other end it has copper deposited upon it. We must rather imagine assimilation and dissimilation as two closely interwoven processes which constitute the still unknown metabolism of living matter, and which *take place simultaneously in even the smallest particles of living matter*, for living matter represents not something fixed or resting, but something more or less labile."

The investigations of HERING on the sensations of light and color led him to the assumption of three kinds of antagonistic processes in the visual substance, corre-sponding with the three pairs of sensations, red-green, yellow-blue, and black-white. The red-green and the yellow-blue reactions each constitute a pair of antagonists which mutually destroy each other, so that only the simultaneous black-white reaction remains. Red and

green or yellow and blue cannot be perceived at the
same time, while black and white can be perceived simul-
taneously and can be mixed in different proportions in
the gray sensations. That we are dealing in this case
with two kinds of antagonistic processes has been suf-
ficiently emphasized by HERING.

MACH has also pointed out in a general way the
essential difference between the two kinds of antagonistic
processes in his doctrine of the sensations of motion.
While criticising PLATEAU'S oscillation theory MACH
writes:

" When two things, *A* and *B*, are designated as positive
and negative with regard to each other, one understands
thereby that *A* can, through the addition of *B*, be in
part or entirely destroyed. This relation exists between
many sensations and their after-images, but not be-
tween all. It exists, for example, between the percep-
tion of a movement and its after-image, which is an
entirely similar movement, but of an opposite character.
It does not exist, however, between the sensations black
and white, of which the one may also be the after-image
of the other. Both sensations are entirely different from
each other, and the two together do not annihilate each
other, but produce, as do two different colors, a mixed
color, namely, gray. In this case, therefore, the terms
positive and negative are not appropriately applied."

According to our conception, those antagonistic reactions
that behave as positive and negative values are homo-
drome, the other heterodrome reactions.

Nothing stands in the way of regarding the individual
kinds of light as catalyzers of antagonistic reactions.
Corresponding with this idea, we would pronounce the

red-green reaction and the yellow-blue reaction as homodrome antagonistic ones, the mobile equilibria of which can be pushed in opposite directions under the influence of two catalyzers. But these reactions may at one time take place in the one direction, at another in the opposite direction, so that red and green can never be simultaneously discovered in a color, no more than blue and yellow. The black-white sensation would, on the other hand, represent a heterodrome antagonistic reaction which, under the influence of white light, moves along one course, but endeavors spontaneously to move back along another. As these oppositely running components of the antagonistic reaction can occur side by side, black and white can be perceived simultaneously.

With this we will bring our consideration of antagonistic reactions in living matter to an end. A more detailed study of the questions which are involved is reserved for a future paper.

If, in conclusion, we look back once more over the path that has been traversed, every step seems to indicate that physico-chemical investigations of a substance that is closely related to living matter are able to throw much light upon the conditions that exist in living matter. In fact, we see that the experimental results obtained in this way pass over to meet those which direct observation of the changes that go on in living matter yields.

Investigations of this kind are well suited to show how all biological methods are of the same value, in that they leave no room for strictly mechanistic or vitalistic tendencies. They form a mighty support for the true scientific monism. The investigator, however, they fill with a conception of those overpowering feelings of the

explorer who imagines himself upon an island until he discovers a connection with the scarcely measurable continent beyond.

4. Therapeutic Studies on Ions.*

> "The acquisition of a new truth is like the acquisition of a new sense, which renders a man capable of perceiving and recognizing a large number of phenomena that are invisible and hidden from another, as they were from him originally."—*Chemische Briefe.*

WITH scarcely more fitting words than these of the old master LIEBIG can we characterize the increase in scientific knowledge and the establishment of new methods of work and points of view for which we are indebted to the application of the laws of physical chemistry to questions in biology and even in practical medicine. If we try to determine, from the varied and many advances that have been made, along what lines these were made, it can be easily shown that the application of three especially of the fundamental laws of physical chemistry to biological problems has been most fruitful. These are the *law of chemical mass action* of GULDBERG and WAAGE, which permits of an insight into the course, the velocity, and the ultimate equilibrium of chemical reactions; the *theory of osmotic pressure*, which has discovered to us the common properties possessed by any series of solutions independently of the nature of the dissolved substances; and the *theory of the electrolytic dissociation of certain dissolved substances*, the foundation of which resides

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* From Munchener medizinalische Wochenschrift, 1903, p. 153. Address delivered before the K. k. Gesellschaft der Aerzte, Vienna.

in the partial dissociation into electrically charged ions which salts, acids and bases suffer when dissolved in certain solvents, especially water.

The effects of salts within and without the organism must all be treated from the standpoint of how far the electrically charged ions or the electrically neutral molecules play a part. Such an investigation, carried out from both a theoretical and a practical point of view, is to form the subject of my address to-day.

I.

It is evident that there exist *different* ways in which the unknown pharmacological properties of a substance may be studied. As least practical and economical would to-day be the attempt to employ the substance directly in cases of illness. The animal experiment is little better, and is of use only when it is possible to reproduce the disease artificially in a more or less perfect way, as in the case of the infectious diseases. Modern pharmacology has been very successful in predicting the nature of the therapeutic effect of newly discovered chemical compounds from their chemical constitution. There exists, however, another way which, though still but little used, renders it possible under suitable conditions to discover unsuspected pharmacodynamic relations. This is the application of a principle which I published years ago, and which has since then rendered possible the solution of difficult problems in physiology. This may be called *the principle of the manifold analogies which exist between the changes in state suffered by colloids and the changes that take place in living matter.* In

attempting to apply this principle, let us discuss first of all the mutual effects of proteins and salts upon each other.

As is well known, proteins suffer a change in state in the presence of many salts—they are precipitated in solid form. In the case of the salts of the alkali metals and magnesium this precipitation does not occur until a certain, fairly high concentration has been reached. The precipitate redissolves when the solution is diluted; the process is, in other words, reversible. We will discuss first of all the laws governing these reversible precipitations.

If the salts are arranged according to their power of precipitating protein (as determined by the use of chemically equivalent solutions), the following table is obtained. + indicates that the protein is precipitated, — that it is not, *n. s.* that the salt has not been studied.

Cations. The precipitating power increases→

		1 Mg	2 NH$_4$	3 K	4 Na	5 Li
I.	Fluoride........	n. s.	+	+	+	n. s.
II.	Sulphate.......	+	+	+	+	+
III.	Phosphate......	n. s.	+	+	+	n. s.
IV.	Citrate........	n. s.	+	+	+	n. s.
V.	Tartrate........	n. s.	+	+	+	n. s.
VI.	Acetate........	—	—	+	+	n. s.
VII.	Chloride........	—	—	+	+	+
VIII.	Nitrate.........	—	—	—	+	+
IX.	Bromide........	—	—	—	—	+
X.	Iodide.........	n. s.	—	—	—	n. s.
XI.	Sulphocyanate...	—	—	—	—	n. s.

The inhibiting power decreases

Anions.

For one and the same anion the precipitating power **increases** from magnesium toward lithium, and for each

metallic ion the precipitating power decreases in the direction from fluoride toward bromide. No matter which anion we take, therefore, we find that the cations always follow the same order when arranged according to their precipitating power, and, conversely, that with any cation the anions always follow the same order when they are arranged according to their precipitating power. This law may also be stated thus: The precipitating power of a salt is determined by the sum of the powers of its individual ions, which act in large part independently of each other.

In what way, now, do the anions and the cations act? It might be thought, first of all, that both the metallic and the acid ions of a salt have a specific precipitating effect, and that the sum of these two positive values constitutes the precipitating power of an electrolyte. According to this conception, the precipitating power of sodium acetate would be made up of the precipitating effect of the sodium ion plus that of the acetic acid ion. Many salts are known, however, which in spite of their ready solubility precipitate protein in no concentration, so that the above explanation does not hold at all in these cases. Ammonium acetate, for example, does not precipitate protein in any concentration, while the acetic acid ion of sodium acetate and the ammonium ion of ammonium sulphate are both strong precipitants. An explanation without contradictions is offered by the following experimentally supported conception. Only the metallic ions, or cations, act as the precipitating constituents of the salts, and the oppositely charged anions inhibit this precipitating property.

It is self-evident that only those salts will precipitate

protein in which the precipitating power of the cations exceeds the inhibiting effects of the anions. Let us study the above table with this idea in mind. The table shows along the horizontal the metallic ions arranged in the order of their precipitating power, along the vertical the anions arranged in the order of their inhibiting effect. It is now at once intelligible why sodium nitrate, with its powerful precipitating sodium ion, coagulates protein, while the weaker precipitants, K, NH_4, and Mg ions, are overcome in their effects by the antagonistic NO_3 ion. Only the lithium salt of the bromides precipitates, and so we might go on.

The table discloses yet other facts. If it is true that the effect of salts upon proteins is determined through the *antagonistic* properties of their ions, then there must exist not only salts which precipitate protein or are indifferent, but also such as prevent precipitation or dissolve already existing precipitates. Observation has confirmed this important conclusion. When it has been determined experimentally that a certain salt behaves indifferently toward protein, then it will be found that all other salts found to the right and above the point occupied by this salt in the table will have precipitating properties, while salts found to the left and below this point will inhibit protein precipitation. The fact that it has been the protein-precipitating salts which have until now chiefly held the attention of investigators has prevented the recognition of this conspicuous phenomenon.

An experiment will best illustrate what has been said. Each of this series of test-tubes contains the same amount of solvent, and neutral potassium tartrate in sufficient

amount to precipitate protein. With the exception of the first tube, they all contain in addition different ammonium salts in chemically equivalent concentrations arranged in the order given in the table. As can be readily seen, the fluoride and sulphate increase the precipitate, the chloride is practically indifferent, while the bromide, iodide, and sulphocyanate hinder in increasing degree the coagulation through the potassium tartrate.

From a large number of similar experiments it has been possible to deduce the following laws, which will be utilized as the foundation for what is to follow:

1. The effect of a salt upon a protein is made up, in the main, of the algebraic sum of the effects of the individual ions.

2. Anions and cations antagonize each other—the cations precipitate, the anions inhibit precipitation; there results in this way a definite grouping of the ions according to the intensity of their actions.

I wish to add that an analogous table of ions has been constructed for the salts of the alkaline earths and the heavy metals, though an investigation of the protein precipitates brought about by these salts is attended by many complicating circumstances.

II.

With the exception of the beautiful studies of DRESER on the toxicity of mercury salts and scattered investigations on the effects of electrolytes on unicellular organisms, there exist but few attempts to apply the ionic theory to the pharmacology of salts. One cannot even say that the conception of the general effects of salts the basis

of which is often sought in the theory of osmotic pressure is entirely clear. The introduction of the ionic theory may perhaps be the first means of bringing a better understanding into this field also. Under these conditions it is readily intelligible that from a classification of the ions won through a study of the changes in state of proteins only broad generalizations can be made. These have, however, shown themselves valuable in pointing out the direction which further investigations must take.

The relation between the cathartic effects of salts and their power to precipitate protein has been recognized for a long time, more especially through the fundamental investigations of HOFMEISTER. If we bear in mind what has been said above, that the power of precipitating protein is a property of the metallic ions, then we will also have to attribute the transitory purgative action of the alkali salts to their cations. This faculty is, in fact, seen to reappear, only accentuated to an extreme, in the case of the heavy metals, which, it is well known, are able to coagulate protein in even the weakest concentration. The mild contractile and purgative action corresponds in the latter case to the erosion and severe gastro-enteritis of the toxic picture. Most of the metallic ions also influence more or less markedly the irritability of the nervous system, the heavy metals producing inflammation and degeneration.

The anions also appear as mighty carriers of pharmacological properties. This is especially true of the end members of one series—the nitrates, bromides, and iodides. They are all readily absorbed and bring about a lowering of the blood-pressure. The Br ion shows a much-utilized sedative action, while the I ion belongs among

those therapeutic agents that have a multiplicity of effects. Besides its specific relation to the metabolism of the thyroid gland, it is employed for its power of lowering blood-pressure in arteriosclerosis and for its absorptive effect upon the most varied products of chronic inflammation, more especially the late forms of syphilis.

Readily as one can recognize in these general considerations how the effects of the ions of a salt are independent of each other, and how there exists an antagonism between cations and anions, the grouping given above suggests something which leads us still further. Even if we proceed most carefully in the extension of our analogy, it must be apparent to every one that the sulphocyanate ion, which antagonizes precipitation most powerfully, constitutes the end member of a series of pharmacologically most active substances, and so the question arises whether this ion does not possess some peculiar medicinal effect. The direction in which such an effect is to be sought is also indicated by the position of this ion in our scale.

III.

So far as I know, there exist no therapeutic experiments on sulphocyanate compounds. At any rate, nothing definite can be found in large handbooks on therapy or in very complete text-books on pharmacology. The rôle of sulphocyanate in metabolism has absorbed the attention of many investigators since its discovery as a normal constituent of the saliva by TREVIRANUS, TIEDEMANN and GMEIIN. As GSCHEIDLEN and MUNK discovered independently of each other, sulphocyanate

occurs also in the urine. The sulphocyanate of the urine might well, however, be absorbed from the saliva, for, according to GSCHEIDLEN, it disappears entirely from the urine of the dog as soon as the excretory ducts of the salivary glands are turned outward.

As to where in the body sulphocyanate is formed, is still unsettled; we know something, however, of the manner in which it is produced. S. LANG has shown that all cyanides and nitrils are converted into sulphocyanates in the organism. This change probably occurs through a process of association with the neutral sulphur of the proteins, for I succeeded in bringing about such a synthesis years ago in HOFMEISTER's laboratory with proteins and their derivatives, provided they had not first been robbed of their readily removable sulphur group.

Recent experiments of BRUYLANTS indicate that a relation exists between the metabolism of the sulphocyanates and that of the purin bodies. In an investigation characterized by originality and far-sightedness, and carried on in 1853 in the Wiener Institut für pathologische Chemie, KLETZINSKY made valuable contributions to our knowledge of the excretion of sulphocyanate in healthy and diseased su jects, which have in the main been corroborated, most recently by GROBER. The intoxication after large subcutaneous doses of sulphocyanate was studied twenty-seven years ago by PASCHKIS on dogs, rabbits, and frogs. Valuable experiments on the effect of sulphocyanate on the metabolism of man and animals have in recent years been made by TREUPEL and EDINGER. MUCK recently discovered sulphocyanate as a separate secretory product in the conjunctival and olfactory secretions.

Of primary interest for us is the proof that iodine and sulphocyanate are excreted in the same places in the organism, that sulphocyanates do not pass through the body in even small doses without altering the metabolism, and that a single dose of sulphocyanate, as discovered by MUNK in 1877, still brings about an increased secretion of this substance a week later. As was recognized by even the earliest workers in this field, the excretion of sulphocyanate is decreased when iodine is taken, and completely stopped when iodism is produced. When this condition exists, GROBER found that even concentrated saliva gives no sulphocyanate reaction.

We are acquainted, therefore, with isolated facts in the physiology and the pathology of sulphocyanate excretion which indicate that a relationship exists between sulphocyanates and iodides.

IV.

The following report on therapeutic experiments with sulphocyanate ions is based upon a rather small amount of material. If we exclude the orientation experiments to determine how much of the substance can be borne and how it is excreted, there are thirty-five cases in all in which careful observations were made and registered, the possibility that other curative agencies were simultaneously active taken into consideration, and these results controlled as far as possible by omitting the sulphocyanate or using other remedies. Since sodium ions are, as far as we know, the most indifferent of the metallic ions from a pharmacological standpoint, sodium sulphocyanate was employed, and this in the maximal

daily dose of one gram. The excretion of the sulpho-cyanate was studied in the saliva and urine.

The existence of a sedative action was tested on a series of neuroses and organic nervous diseases in which the signs of an increased excitability, such as fear, irritability, sleeplessness, increased reflexes, tremors, etc., existed. Ten cases in all were studied: 2 cardiac neuroses, with signs of general neurasthenia, 3 neurasthenics, 2 general paretics, and 1 tabes dorsalis, with increased irritability, and 2 climacteric neuroses. In nine of the cases positive results were obtained. Within two to five days the patients became much quieter, and with further use of the drug a very decided improvement in even the most disturbing symptoms took place. The fear, restless sleep, headache, and dizziness became less, the troublesome congestions of the climacteric women passed away, and a sense of rest repeatedly took the place of weariness in the patient. Only in one case did the original neurasthenic symptoms return after being absent for some days. These were connected with periodic pains in the splenic region, which radiated toward the epigastrium, the pathology of which remained obscure. A subsequent use of bromides was also without effect in this case. The fact that the symptoms of increased excitability in organic and functional nervous diseases began to disappear a short time after taking sulpho-cyanate and continued to improve as time went on, that the old symptoms reappeared when the drug was no longer given, especially when it was taken away shortly after its use had been begun, that previous indifferent methods of treatment had brought no great change in the patients' condition, and that, finally, unprejudiced

observers as well as the patients themselves felt convinced
of the immediate good effects of the drug, seems to justify
the conclusion that sulphocyanate possesses, as a rule, a
well-marked sedative action upon the pathologically
excited nervous system.

The experiments with sulphocyanates were then ex-
tended to a group of diseases which have one symptom
in common, namely, an increase in the blood-pressure.
These included arteriosclerosis, aortic insufficiency, and
chronic nephritis.   From the large number of patients
with circulatory disturbances in Professor v. BASCH's
wards, eleven cases of arteriosclerosis were chosen, nine of
which showed tortuosity and great pulsation of the blood-
vessels, hypertrophy of the left ventricle, accentuation of
the second aortic sound (usually with an increase in the
intensity of the apex-beat), and marked increase in the
blood-pressure, besides a number of subjective symptoms.
These consisted of pains in the chest, a feeling of pressure
and shortness of breath on exertion, especially after meals
or at night, a sense of fear and disturbed sleep, while
two of them showed, in addition, attacks of dizziness
and ringing in the ears.

Corresponding with the uniformity in clinical material,
the drug also showed a uniformity in action.   The sense
of fear disappeared; the attacks diminished within a few
days, to return later only under special provocation.
Disturbances which had existed for months, such as
ringing in the ears and attacks of dizziness disappeared,
not to return.   In most of the patients systematic blood-
pressure determinations were made with v. BASCH's
sphygmomanometer by Dr. S. KORNFELD, who very
generously gave me the benefit of his years of experience

in this field. A steady drop in blood-pressure, amounting from 10 to 25 per cent. of the original value, could be ob served within a few days in all the cases studied. As soon as the drug was stopped the blood-pressure rose again, often to the original height within three to four days. Accompanying this, there was also a return of some of the previous symptoms. Of interest are two further cases of sclerosis of the larger arteries. One of these was a female patient with sclerosis of the abdominal aorta, which could with its large branches be readily palpated and which gave rise to severe attacks of pain in the abdomen; the other, a woman with all the signs of an advanced arteriosclerosis, who had suffered for months with pain and weakness in the right arm, which appeared on the slightest exertion or when the arm was allowed to hang by the side for some time. Besides this there existed a so intense and painful sense of cold in the diseased extremity, which corresponded with a very noticeable decrease in the temperature of the skin, that the lower arm and the right hand had always to be wrapped in thick cloths. After a test had been made with sodium bromide and aspirin, and these had proved entirely without effect, the pains diminished very markedly after an eight days' use of diuretin. After taking sodium sulpho-cyanate for a number of weeks the sensation of cold gradually disappeared, the hand no longer needed to be wrapped in cloths, and the weakness in the extremity disappeared sufficiently so that the patient, who had been under observation for eight months, was able to do light work about the house. The blood-pressure fell during this time from 210 to 160 mm. of mercury. The patient with arteriosclerotic pains in the splanchnic

region was much relieved, especially during the first weeks, by the sulphocyanate treatment, while other methods had been without effect. Whenever the sulpho-cyanate administration was interrupted, the blood-pressure rose, and with it returned the old symptoms.

Besides the decrease in the blood-pressure, a sedative action may also play a rôle in the medicinal effects of sulphocyanates. It is possible that yet another factor plays a rôle, which can, however, only be touched upon here. In investigations carried on during the past two years with Dr. PETER RONA on the relation between the effects of iodides and sulphocyanates and the effects of salts of the heavy metals and the alkaline earths, the following has been found:

It has been definitely established through exact observation and experiment that the iodides bring about and favor the excretion of the ions of the heavy metals, such as lead and mercury, in cases of chronic intoxication. The explanation of this fact, which has been utilized therapeutically, has been sought in the formation of soluble albuminates, which the iodides have been supposed to bring about.

When quantitative experimens on protein precipitation are made, it is found that the heavy metals show at first a rapid increase in the precipitation value, with an increase in the concentration of the salt, which later, however, as in the case of zinc, gradually falls to zero. When the concentration of the salt is still further increased, a precipitate appears a second time, which is very heavy and which also is again soluble. Curves in which the ordinates indicate the degree of precipitating power, the abscissas the amount of salt of a heavy metal employed,

can be constructed to give a graphic survey of these relations. Such precipitation curves suffer a great change upon the addition of iodides or sulphocyanates. If the ions of the heavy metals are present in a low concentration, the precipitation of the protein is prevented altogether; if present in larger amounts, the protein precipitation is markedly increased. In the case of living animals only the former possibility, that of the presence of but a low concentration of poisonous ions, comes into consideration. The iodides and still more the sulphocyanates do in fact act under these circumstances as substances which favor the formation of readily soluble protein compounds, through which an elimination of the heavy metals is greatly aided. Conditions in the test-tube and in the animal body are here, in the main, identical.

The relation of the metals calcium, barium, and strontium to the proteins is somewhat different, a fact of interest because of the important rôle of the calcium ions in physiological and pathological questions. These metals stand in many ways between the alkali metals and the true heavy metals. They have, in common with the former, a high precipitation limit; with the latter, the fact that the combination between protein and metal is a firm one, and once produced continues even upon the addition of water to the solution. The power of depressing most markedly the precipitation limit of the earthy metals is possessed to a slight degree by the Br ion, in greater degree by the I ion, and most powerfully by the sulphocyanate ion. In the presence of sulphocyanate ions, calcium, strontium, and barium chloride will precipitate protein when present in simple normal solution and even below this, while under ordinary circumstances the two

latter will not coagulate in any concentration, and calcium chloride only when present in nine times the concentration given above. This formation of a solid protein compound is always preceded by a state in which an intimate combination between the protein and the metallic ion occurs even before the precipitation limit is reached. It can readily be seen that, through the establishment of a firmer combination between protein and calcium ions in the presence of a few iodide or sulphocyanate ions, the formation of other insoluble calcium salts can be inhibited, and the excretion of calcium be increased in this way. The therapy of arteriosclerosis has within recent years been directed in no small degree against the calcification process itself. It seems to me that what has been said above serves as a theoretical basis for the clinical experience of the best observers in this field, that the continued use of iodides is able to retard the course of arteriosclerosis. For reasons which will become clearer later, the use of sulphocyanates in these cases would represent a therapeutic advance.

To the series of cases of arteriosclerosis there belong two cases of aortic insufficiency with palpitation, dizziness, headache, a feeling of fear, and increased blood-pressure. In one of these permanent relief from all symptoms was achieved, in the other a temporary one, as the therapy had to be interrupted after eight days.

Four patients with chronic Bright's disease, hypertrophy of the heart, and pallor showed a rapid betterment of their subjective symptoms, consisting of backache, neuralgias, and sleeplessness, when put on a milk diet and sulphocyanate. Without attributing the betterment to the sulphocyanate, it could be shown in these

cases that an existing chronic albuminuria does not contraindicate the use of the drug.

My last observations are taken from a group of patients with syphilitic headaches—two men and two women. In the men there was a history of syphilitic infection; in the women abortions and still-births had occurred. One man and one woman had a year previously suffered from the same symptoms, which had promptly disappeared after the use of iodides, and only after these. The headaches were in all cases very severe, and in three of them typically nocturnal in character, particularly during the weeks when this symptom first developed. Sensitiveness of the skull upon percussion also existed.

The syphilitic character of the pains was therefore definitely established in these cases, so far as this is clinically possible. This diagnosis was further strengthened by the uselessness clinically of physical healing methods and antineuralgic remedies.

The effect of the sulphocyanate in these cases was so prompt and so clearly beneficent, even in the first few days after administration, that its medicinal properties cannot be doubted.* The effect upon the patients proved in all cases to be a lasting one. The two patients who had for similar symptoms previously undergone a treatment with iodides agreed that the feeling of relief had this time come much more promptly. In one of the patients, who could tolerate drugs only badly and who had a year previously suffered from severe iodism,

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* I do not, of course, on the basis of these few observations on a *special* form of late syphilis, want to look upon the question of the specific effects of a sulphocyanate therapy in syphilis as settled.

the sulphocyanate was administered in two small enemas daily.

This constitutes a summary of my experiences thus far. They were obtained for purposes of general orientation on patients of whom each group presented a great, almost monotonous similarity in symptoms. When the careful choice of cases is taken into consideration, it can scarcely seem strange that the effect of the drug should have been so uniform. In passing it must have been apparent what small doses of sodium sulphocyanate proved remedially effective. The explanation of this fact lies in part in the low molecular weight of the substance, for 1.0 gram sodium sulphocyanate is equal to 2.15 grams sodium bromide or 2.82 grams sodium iodide.

But a close relationship between these substances shows itself not only in the harmony of their advantages, but also in the harmony of their disadvantages. There exists a sulphocyanate acne and a sulphocyanate rhinitis. The latter is the commoner. Twice a mere suggestion of it occurred, in one of these cases only once toward evening. In two other cases copious secretion from the nose and eyes set in, which disappeared, however, within two to three days after the administration of sulphocyanate was stopped. In only one patient with a sensitive skin, which had some months previously been the seat of a bromide exanthem, a sulphocyanate acne appeared, consisting of small nodules, most numerous in the face and sparingly distributed over the trunk and extremities, which did not, however, give any further trouble. It disappeared a few days after the medication was stopped. Gastric disturbances never appeared, even

after the remedy had been used for months. While a great similarity, in part even an identity, seems to exist between the iodides and the sulphocyanates, the latter do not possess the specific effect upon the thyroid gland. The use of the drug even for months does not affect a parenchymatous goitre. This fact might well at times prove of therapeutic advantage.

Therapeutics still continues to count the salts among the " alterants" and the " resolvents." Both of these properties of " changing the course of a disease" and " bringing about a resolution" the sulphocyanate ions possess in rich part, as shown by our own observations. From the versatility and intensity of their action we are presumably correct in putting them beside the iodide ions.

I do not wish to bring this paper to a close without adding a few general remarks on the principle which prompted it.

This leads, first of all, to a conception of the way in which the salts act upon the organism, which, though still hypothetical, encounters at present nothing which speaks against it. Just as a large group of non-ionized narcotics bear an intimate relation, according to H. MEYER'S and OVERTON'S beautiful discoveries, to the lipoids of the cell, the ionized compounds might find their point of attack in the protein constituents of the protoplasm. A difference in the distribution, or a replacement of the normal ions of the cell, would be connected with changes in the state of the colloids and consequently also changes in function. If the application of our principle extends, on the one hand, almost to the chain of psychophysical processes, it has its limitations,

on the other, which are inherent in the nature of such a method itself.

When we speak of the great analogy between changes in state in organic colloids and life phenomena, we are dealing, to use the words of MAXWELL, "with that *partial* similarity between the laws of one series of phenomena and those of another, in consequence of which the one comes to illustrate the other." Besides a limited identity, extensive differences may therefore exist, many of which are still beyond our understanding. This holds also for the great differences known to exist in the toxicity of ions in animal experiments. We are at present still much inclined to attribute, qualitatively at least, great importance to the value of animal experiments for settling such a question as the one before us. This we do unjustly, I believe. Clinical therapeutic experience must be gotten by itself. The animal experiment brings in this case also only an analogy; at the best it serves only to stimulate further work.

5. On the Relation between Physico-chemical Properties and Medicinal Effects.*

IN the consideration of questions in pharmacodynamics, from the point of view of physical chemistry, a scarcely measurable field lies before us, into only a small part of which, however, paths lead at present. If under these conditions I attempt to speak before a body of clinical men on some questions which belong in this realm and which have interested me for several years, I

* From Verhandlungen des XXI. Congresses für innere Medicin in Leipzig, 1904.

can give many good reasons for doing so. As is evidenced by the studies of H. MEYER and OVERTON on the theory of narcosis, and by the work of STRAUB on the tenability of the law of mass action for the distribution of certain poisons in the organism, so our work, too, has clearly shown that only the use of physico-chemical methods renders possible a deeper understanding of medicinal effects. To this there comes the well-founded feeling that in this way explanations of the nature of the important phenomena accompanying constitutional changes may also be obtained, a problem which has long been a favorite one with the internal clinicist.

This paper will be limited to a discussion of the rôle of ions, those electrically charged dissociation products of acids, bases, and salts which are produced when these substances are dissolved in water. We know that the mineral constituents of the body are, in the concentration in which they are present, almost completely dissociated. This is true also of the metallic and alkaloidal salts which are introduced into the body for medicinal purposes. Other pharmacologically active substances which scarcely ionize on solution in water, such as the esters, may be converted into ionizable compounds in the body. Because of this many-sided importance of the omnipresent ions, it seemed of value first of all to get an experimentally demonstrable conception of the mode of action of the ions in the animal body. Of all the constituents of the protoplasm, the proteins show the most intimate relation to the salts. Their most varied changes in state, such as solution, precipitation, and coagulation through heat, are all connected with the coöperation of salts, and it seemed necessary therefore to obtain first

of all a more intimate knowledge of the significance of the ions for these changes in state.

The neutral salts of ammonium, magnesium, and the alkali metals are best adapted to such a study, as the protein precipitates produced by them can be reobtained in an almost unaltered condition through dialysis of the salt. The most important laws governing this reversible change in state are the following: If the salts are arranged according to their precipitating power, the acid ions (or anions) always follow each other in the same order with any given metallic ion, and, conversely, with any acid ion the metallic ions (or cations) always follow each other in the same order. The precipitating power of any salt represents, therefore, the product of the effects of its constituent ions, and the properties of these ions are to a large extent independent of each other.

A second law governs the character of the ionic effects. It was found that the ions of a salt antagonize each other in bringing about changes in the physical state of a colloid, for the one ion has a precipitating action, while the other has a solvent action, and, as the one or the other ion has the upper hand, the salt under consideration either precipitates or prevents the precipitation of protein. The effectiveness of ions is expressed in the following series:

Cations arranged in the order of their *precipitating power*. The most powerful comes first: *sodium, potassium, ammonium, magnesium.*

Anions arranged in the order in which they *prevent precipitation*. The most powerful comes last: *sulphate, citrate, tartrate, acetate, chloride, nitrate, bromide, iodide, sulphocyanate.*

To render what follows more intelligible it is necessary

to touch upon the relation of the alkaline earths to the proteins.

The protein precipitates produced through the action of the alkaline earths are irreversible in so far as the solution of a precipitate that has once been produced is difficult and scarcely leads to the restitution of an unchanged protein. The effects of calcium, strontium, and barium are determined to a large extent through the presence of other ions simultaneously present. Under these circumstances the effects of the ions of a salt of an alkali metal also antagonize each other, only in a way opposite to that observed in precipitations produced through pure neutral salts. The formation of a precipitate in a solution of native protein through the alkaline earths is *favored by anions* and *inhibited by cations*. The anions under these circumstances arrange themselves in the following order, in which the most powerful precipitants come first:

Acetate, chloride, nitrate, bromide, iodide, sulphocyanate.

The cations arrange themselves in the following order in which the greatest inhibitor of precipitation is put first:

Magnesium, ammonium, potassium, sodium.

Nothing stands against the assumption that it is the protein constituents of protoplasm that constitute the point of attack of the ions in the organism, and this belief cannot only be supported by experiment, but is also of heuristic value.

The two chief laws of protein precipitation, the additive and the antagonistic effects of ions, hold also for the physiological properties of ions. All cations, for example, have certain physiological characteristics in common, in

that they increase more or less the irritability of nerves and muscles, excite intestinal activity even to the point of producing a gastro-enteritis, and usually increase blood-pressure. The solvent action upon protein and, running parallel with it, the physiological effects of the anions are the more pronounced the further we pass along the series of anions given above. The protein precipitating salts, such as the sulphates, citrates, and tartrates, when these are connected with the overbalancing properties of the metallic ions, are all cathartics. The salts which follow these, more especially the nitrates, bromides, iodides, and sulphocyanates, show the characteristics of the anions; they are sedative in their action and decrease blood-pressure. The relationship between them is indicated also in the similarity with which they bring about an acne and coryza. For the sulphocyanates these pharmacological properties were deduced from the grouping given above and verified on patients.

The experimental study of the effects of sulphocyanates has proved fruitful in yet another way, in that the sulphocyanate anions, representing as they do the last members of the anion series, allow one to study the properties of anions in a particularly pure form. Sulphocyanates can be used first of all in order to ascertain more accurately the *physiological relations between salts and esters*. Between the strongly ionized salts and the scarcely dissociable esters (which represent combinations between alcohols and acids) there exists a great difference in their power of penetrating a cell. While the latter, according to the extensive investigations of OVERTON, readily enter a cell because of their solubility in its lipoids, —lecithin, cholesterin, cerebrin, etc.,—the salts enter the

protoplasm only with difficulty. Since, however, the esters are saponified in the organism, in consequence of which the anions of the acids become free, a physiological ion effect might nevertheless be expected under favorable conditions, even after the administration of esters. Such an effect will become apparent, however, only when in a readily dissociated ester some anion is present that has characteristic physiological properties and is sufficiently active physiologically to show itself, otherwise the narcotic and circulatory effects common to all esters conceal the intoxication picture. If for purposes of comparison experiments are made with sodium sulphocyanate and the amyl ester of sulphocyanic acid, *a typical sulphocyanate intoxication occurs in both cases.* Careful analysis shows that this consists in a fall in blood-pressure brought about through a decrease in the energy of the heart muscle, a subsequent increase in pressure through stimulation of the vascular centres and great, principally central, stimulation of the vagus. While, however, the intravenous injection of two to three drops of the ester suffice to bring about a rapid and fatal intoxication in a medium-sized dog, eight to ten grams of sodium sulphocyanate must be introduced intravenously to bring about the same effect. This enormous difference in toxicity shows that in the amyl-sulphocyanate intoxication the ester readily enters the cells, because of its solubility in the lipoids, and that not until it has arrived in the cells are its anions set free. These same ions, when already formed, enter the cells only with difficulty, in consequence of which the body must be charged with a great excess of sodium sulphocyanate in order to bring about the same degree of intoxication,

Numerous instances in pharmacology, in which any alcoholic radicle in an ester-like combination with an acid is required to bring about any specific effect, can, I believe, be explained in the same way. The alcohol radicle only renders possible the ready absorption of the substance by the cell; the anion connected with it is the really active principle. Cocaine is, for example, a methyl ester of benzoylecgonin, a substituted tropincarbonic acid. The benzoylecgonin, the real carrier of the medicinal properties, is however twenty times less poisonous than its ester, cocaine, and does not possess the anæsthetic properties of the latter. Only after being converted into an ester, through any alcohol whatsoever, is the cocaine effect produced. *Existence in the form of an ester is apparently always the sine qua non of a useful local anæsthetic* whose active anion must enter the endings of the sensory nerves. EINHORN has found that a large number of cyclic and heterocyclic esters are able to bring about a local anæsthesia, and has been able to discover valuable substitutes for cocaine in the orthoforms, which represent methyl esters of amido-oxybenzoic acid, and in nirvanin, a diethylglycocoll compound of orthoform. Eucaine and anæsthesin are also esters, the latter one of p-amido-benzoic acid. Without being directly concerned in the physiological effect produced, the presence of an alcohol radicle in the compound first renders such an effect possible, for only under these circumstances is the active acid ion present in sufficient concentration at its point of physiological attack. Arecaidin, which chemically represents a methyltetrahydronicotinic acid, is scarcely active physiologically, while its methyl ester, arecolin, represents the toxic principle of the areca-nut.

The number of these illustrations might easily be multiplied. In passing I only wish to point out that the activity of the metallic ions can be increased through combination with an alcohol radicle' in the same way as the activity of the acid ions. It is possible in this way to bring about in animals most acute metallic intoxications with the ethylic compounds of zinc, mercury, and lead.

Besides this influence upon the effects of ions, brought about through combination with alcoholic radicles, dependent upon the fact that protoplasm is made up of lipoidal and proteoidal material, there still exists another way in which the specific effects of many ions can be increased or decreased, namely, through combination with other ions. As an example of this we may take the behavior of the alkaline earths toward protein in the presence of neutral salts of the alkali metals. As a glance at the lists of ions given above shows, the protein precipitations brought about through the alkaline earths can be inhibited through the addition of ions of the alkali metals, or hastened through the addition of anions, most powerful of which is the sulphocyanate anion. It seemed possible, therefore, that through proper experiments a *physiological antagonism* could be discovered to exist between monovalent cations and the alkaline earths, as well as a *synergistic increase* in the effect of anions through the cations calcium, strontium, and barium. This suspicion, prompted by analogies existing between experiments carried on in vitro and certain phenomena observed in vivo, could be shown to be correct by animal experiments.

If animals are kept in a half-intoxicated state with

sulphocyanate—a state which is characterized by a strong, steady heart action and stimulation of the vagus nerve and vascular centres—it is possible to bring about an immediate standstill of the heart, not preceded by an increase in blood-pressure, by injecting an amount of a barium salt which, under ordinary circumstances, scarcely affects the heart at all, or, if it does, only stimulates it. Such a heart-failure may under circumstances occur after the injection of five milligrams of barium chloride into a medium-sized dog. Since this effect can be obtained even in a completely atropinized heart, or after the exclusion of the greater circulation and the nerve-centres, it is probable that the salt affects the heart directly. Just as in test-tube experiments with protein, barium, which is characterized by a remarkable affinity for the musculature of the heart and the blood-vessels, is able to join such large amounts of neighboring sulphocyanate ions to the heart muscle in such remarkably short time that an acute, deadly sulphocyanate intoxication results. How small amounts of sulphocyanate suffice in order to bring about a heart failure, if only it reaches its point of attack within the cells, was indicated in the experiments described above with sulphocyanate esters. Calcium and strontium act in the same way as barium, only, because of their lesser affinity for the musculature of the heart, larger doses are required (PAULI and A. FRÖHLICH).

The *physiological antagonism* between many ions which we supposed to exist from a certain parallelism between the behavior of dead and of living protein, and which has been proved to exist experimentally, had been previously discovered in another way by the well-known American

physiologist J. LOEB, and rediscovered under the most varied conditions by his numerous pupils. We can only touch upon these investigations here. That they harmonize with our own findings and represent only an expression of the general principle common to them all is, however, readily discernible. The starting-point of LOEB'S investigations is the question of the significance of the different ions of sea-water for the life processes of marine animals. A great similarity was found to exist between the effects of various ions upon phenomena of development and upon the activities of muscle and nerve. An interesting example is furnished by the development of the eggs of *Fundulus*, a small bony fish. These fish are able to develop not only in sea-water but also in distilled water. If immediately after fertilization these eggs are introduced into a sodium chloride solution of the concentration of the sea-water they all die in the course of a few hours without developing any further. If, however, a small amount of calcium, which also represents a constituent of the sea-water, is added to the sodium chloride solution, normal embryos are produced. A pure sodium chloride solution is poisonous also for the adult animals, but this toxicity, too, is done away with upon the addition of a little calcium. Instructive also are the effects of ions on the rhythmical contractions of the swimming-bell of medusæ. After removal of the central nervous system in these animals the bell still contracts rhythmically in a pure sodium chloride solution. These contractions cease, however, as soon as certain cations, such as calcium and strontium, are added to the solution, just as they cease in sea-water. In a similar way, the poisonous effects of pure sodium chloride on

heart muscle or its stimulating effects upon the excised gastrocnemius of the frog are also done away with through the addition of calcium or strontium.

RALPH S. LILLIE demonstrated an analogous antagonism between the effects of cations on ciliary movement. MacCALLUM showed in animal experiments that the effect of cathartic sodium salts, which is to be looked upon as an expression of the activity of the metallic ions, can be inhibited through administration of calcium. MARTIN H. FISCHER was able to demonstrate that a glycosuria, brought about through the injection of sodium salts into rabbits, can be suppressed through calcium, and, according to BROWN who discovered the same fact independently, also through strontium.

That the relations between anions are governed by similar laws within and without the organism is apparent from the investigations of TORALD SOLLMANN. As this author was able to show in his studies on diuresis, the urinary excretion of chlorine ions from the body under the influence of other ions is to a large extent independent of the amount of water secreted along with them. The per cent. of chlorides in the urine is increased through administration of nitrates, iodides, and sulphocyanates, decreased through acetates, phosphates, and sulphates. It is not difficult to recognize in this grouping the order in which the anions act upon the proteins. The second group acts less powerfully, the former more powerfully than the chlorine ions in the effect of the ions of the alkali metals upon proteins.

After what has been said it will no doubt be admitted that a considerable material is already at hand which lends further support to the *principle of the many analogies*

between changes in the physical state of colloids and the changes which go on in living matter.   It would, however, be unfair to expect such a principle to hold quantitatively for every biological detail.   It is subject rather to the various changes brought about through differences in living matter which vary with different kinds of animals and with different kinds of organs.   While calcium, strontium, and barium have an almost equal effect in the presence of sulphocyanates on egg albumin in a test-tube, the synergistic function of the barium is the most apparent of the three in a physiological experiment on the heart.   In a similar way LOEB and HERBST found that individual differences exist between ions in their effect upon different marine animals.   Nevertheless, the general law governing all these phenomena appears everywhere, at one time more, at another time less distinctly, just as in a musical composition the theme is heard at all times by him who has once learned it among the infinite number of its variations.

6. Changes Wrought in Pathology through Advances in Physical Chemistry.*

NOT until it has been found possible to explain the anomalies in the functions of the organism through changes in the form and in the composition of its constituents can pathology consider its task completed.   Its field of knowledge grows in three ways: through the experi-

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* Wandlungen in der Pathologie durch die Fortschritte der allgemeinen Chemie, Wien, 1905.   Festival address at the third annual meeting of the K. k. Gesellschaft der Aerzte in Vienna, March 24, 1905.

mental determination of the degree and the direction of changes in function; through a study of the morphological and through a study of the chemical deviations from the normal lying at the basis of these changes in function. When one studies the development and the present status of pathology one soon sees that this science has not grown with the same rapidity, nor equally well in all three directions. It has developed most markedly toward the morphological side, least of all toward the chemical side. This difference is still more apparent when we study more particularly the part that the Vienna School has played during the past century in the development of pathology. Chemical methods have scarcely at all been employed, except in the last ten years, while the morphological investigations carried on in Vienna during this same century have been truly brilliant.

It may not be without interest to touch upon some of the reasons that have brought about such a noticeable difference in the pursuit of these two branches in our school. It was, of course, but natural that the conceptions of the famous men of the second great period of the Vienna School should have determined for a long time to come the course which further development in the past century took. If this development was chiefly morphological in character, then this depended in large measure upon the state of chemistry in Austria at that time. For the education and the first efforts of those pioneers occurred during an epoch when chemistry was most deplorably represented in Austria, followed and taught as it was, not experimentally, but speculatively. A classical document describes conditions as they were in those days. In one of his memorable addresses LIEBIG,

in 1837, so frankly and mercilessly criticised the condition of chemistry in Austria that it seems small wonder that the succeeding generation of medical men, mightily influenced through the labors of such as CORVISART, LAENNEC, and BRÉTONNEAU, dedicated themselves chiefly to morphology, which promised so much.   The experiences of medicine with a chemistry which had been in the main of a speculative character no doubt also contributed to this end.   The birth of iatro-chemistry, which through PARACELSUS, HELMONT, and SYLVIUS ruled medicine in the seventeenth century, met a just and fruitful opposition through the morphological workers.   Later BOERHAVE, with whose entry the first brilliant epoch in medicine is intimately connected, most emphatically emphasized the great importance of chemical research in pathology, but because of its insufficient development chemistry could offer too little at that time to fulfil his promises.

Not until the wonderful development of organic and applied chemistry as introduced in the middle of the last century, more especially by LIEBIG, did new paths open up before pathology.   In the meantime, however, the morphological tendency in Vienna had, under the tremendous influence of its illustrious exponents, obtained the upper hand, a fact which helped to determine also the nature of the increase in the faculty.   Into the circle of their influence were drawn the majority of the younger men of talent and permanently kept there.   For it resides in the nature of morphological research that it is admirably adapted for the introduction of the beginner to science, in that it offers a large number of simple problems which may be solved without drawing upon a larger number of accessory sciences, while its results, which usually repre-

sent a truthful description of what has been seen, give a feeling of great security.

The far-reaching results of chemical research in the last decade have brought to us also a gradual increase in the *general* interest taken in the chemical aspects of pathology, and so it has been no accident that this altered point of view has found an expression in the change in the character of the annual addresses made before our society. Three years ago we enjoyed an inspiring presentation of the pathology of metabolism, and last year brought us a sharply defined discussion of the protein question, such as only ripe experience, hand in hand with critical judgment, can produce. If I to-day find myself once more face to face with the problem of bringing before you in our annual meeting another chapter from the realm of chemistry as applied to medicine, I must attribute this honor first of all to that great new interest taken in this subject. This subject is, in fact, worthy of your greatest attention, for we stand at present in the midst of an undreamed-of improvement and development in our point of view in physiology and pathology, and this strange and sudden change is based in particular upon advances in general or physical chemistry. This science has acquainted us with a series of fundamental laws that govern chemical reactions, whose validity, influenced more or less through special circumstances, extends also to the changes that go on in living organisms.

It was probably the first important step for the physico-chemical characterization of living matter when GRAHAM divided all bodies into colloids and crystalloids, according

to the behavior of certain typical substances. For from this division there has arisen the conception that all living matter is of necessity connected with the existence of a colloidal ground-substance in which all changes take place. Nevertheless, more than twenty years were necessary before a systematic attempt was made to obtain a conception of the changes that go on in living matter from a study of changes in the state of colloids. In the three years from 1888 to 1891 HOFMEISTER took the first step in this direction, in that, in attempting to explain the physiological effects of salts, he compared the effect of salts upon colloids in a test-tube with the effect of these salts upon the animal organism. During the last decade similar investigations have been carried on with the means offered by physical chemistry, which has during that time enjoyed most rapid growth, and have, in conjunction with the investigations of chemists on inorganic colloids, furnished so abundant a material that the time for a broad alliance between biology and colloidal chemistry seems to have come. From this most promising section of applied chemistry I would to-day like to take a few facts which to all appearances seem to be of fundamental biological importance.

The colloidal substances are present in two forms in the body—in a more or less jelly-like condition in the cells, and in a fluid condition in the blood and the tissue juices. The laws of colloid chemistry govern the changes that go on in the cells, only these laws are modified through the metabolism of living matter, the characteristics of which we do not as yet understand. The behavior of the extracellular material is, however, of a much simpler character. In the former case there exists only

a certain parallelism between changes in colloids and many manifestations of cell life; in the second, however, we are dealing with a direct applicability of the laws of colloid chemistry, controllable at any time by experiment. We will to-day discuss only these last-named phenomena, the importance of which has recently been pushed into the scientific foreground, more especially through the modern development of the teachings of immunity.

Before attempting an explanation of the significance of the phenomena which interest us especially, it is necessary to obtain a clear conception of the characteristic properties of the colloidal condition. These characteristics are best illustrated by the properties of the colloidal solutions of metals, from which a gradual transition to the biologically important colloids occurs.

If a clean metal plate, such as platinum, is put into water it assumes a weak electric—in this case negative—charge, while the fluid surrounding it becomes electropositive. According to the fruitful conceptions which NERNST has developed of the source of galvanic currents, we have to deal with the following process: Just as after the solution of a salt—such as sodium chloride—in water the metallic portion is present in the form of electropositive particles, the acid portion in the form of electronegative particles or ions, a metal when dropped into water also goes into solution in traces to form electropositive metallic ions, while the metal itself becomes a negative electrode. A proper combination of such metals having different solution tensions then constitutes a galvanic element. If now we imagine such a metal divided under water into smaller and smaller particles until this metallic dust is able by virtue of its

minuteness to remain suspended in the liquid an indefinite length of time, then we have a colloidal solution before us. Such a solution is perfectly clear and passes unchanged through the finest filter. We can deduce from the manner of its origin its characteristic properties —such a solution represents a suspension of fine electrically charged particles, in other words minute electrodes. If two gold electrodes connected with a strong electric current are introduced, according to the directions given by BREDIG, into pure water cooled by ice and then are carefully separated until a tiny arc appears, purplish-red clouds begin to emanate from the negative electrode as this goes into solution in the form of fine dust particles, and the result is the production of the beautiful colloidal gold solution which ZSIGMONDY prepared previously by chemical means through careful reduction of a gold chloride solution. Partially through use of the chemical method, partially through use of the electrical method, a large number of inorganic colloids have been produced, which have, since GRAHAM'S fundamental work, formed a much-cherished object of investigation. But not until recently has it been possible to deduce in a satisfactory way the laws governing their varied behavior from variations in a few characteristics. By utilizing the fundamental work of such investigators as LINDER, PICTON, HARDY, and BREDIG, and many of his own experiments, J. BILLITZER, a Vienna chemist, has been able to show that the chief laws governing and the differences existing between colloids can all be explained by variations in only three values, namely, the number, size, and electrical charge of the suspended particles. This conception, which

allows not only a survey of what has been accomplished, but also allows us to state in advance what may be expected to happen in colloidal solutions, has, moreover, the advantage that its suppositions are capable of being tested experimentally—in fact, have already been tested in many cases.

We have optical means at our disposal, for example, by which we can determine the number and the size of the colloidal particles. If a colloidal solution is placed in a bundle of intense light rays, the fine particles of the solution reflect the light in part, as can be determined through its polarization. The absorption of different portions of the spectrum may also give a clue regarding the size of the particles. Finally, SIEDENTOPF and ZSIGMONDY have made it possible with their ingenious ultramicroscope to determine the number and size of the colloidal particles. The electrical condition of the suspended particles we can recognize from their behavior in the electric current, in that they migrate, according to their electrical charge, either toward the positive pole when they are negatively charged, or toward the negative pole when they are positively charged.

These facts allow us to understand a process which has long served as the prototype of most of the colloid reactions and which explains many important questions in physiology and pathology, namely, the precipitation of colloids.

It has already been pointed out that the salts, acids, and bases dissociate in part in aqueous solution into their oppositely charged constituents, the ions. Let us suppose now that a sufficient number of such ions are introduced into a colloidal solution of a metal which

represents a suspension of weakly charged electronegative particles. In consequence of electrical attraction, the negative colloidal particles will collect about the electropositive ions, until through the heaping up of a sufficient number of such particles the collecting ion will be electrically neutralized. When the aggregates thus formed have reached a sufficient size, the solution becomes turbid, and finally a precipitate drops to the bottom.

We are able to foretell the possibilities which result from a change in the number, size, and electrical charge of the particles of a colloid, all of which can be proved by experiment. When the number of particles is decreased, the probability that a sufficient number will be collected together through added ions for the formation of large aggregates is also decreased, and finally a stage is reached in the concentration of the colloid below which precipitation does not occur. The precipitation is also rendered difficult when the particles are very small and carry but a weak charge, because under these circumstances too large a number of the particles have to be collected together. If, on the other hand, the electrical charge of the particles is too great, then too few suffice to neutralize the oppositely charged ions, and the aggregates formed are too small to settle to the bottom. A medium charge, a sufficient number, and a sufficiently large size of the colloidal particles constitute, therefore, the optimum for precipitation. Just as we have electronegative metallic colloids, we have also electropositive colloids. Oppositely charged colloids precipitate each other in the same way as the ions of salts precipitate a colloid, only in consequence of the size of the reacting

particles the conditions for the formation of large aggregates are especially favorable.

We have yet to speak of a few typical observations on the precipitation of colloids that have become of great importance for certain questions in biology. Such, for example, is the often-observed *variability in colloids*. This variability often does not attain a stabile end state until after a very long time. It has been found that this original instability is dependent chiefly upon the presence of impurities introduced during the preparation of the colloid, which because of their slight amount do not make themselves felt until a long time has passed. As soon as this slow process of neutralization has come to an end, the colloid is in a stabile condition.

A further very important observation is the great influence that *time* has upon the formation of a precipitate. We usually require very different amounts of a precipitating salt or a colloid, depending upon whether the precipitate is to be brought down at once or more slowly. Not rarely the amount of precipitating substance used in the second case is larger than in the former. This is dependent upon the following fact: If the precipitating colloid *A* is at once added to the colloid B, the particles are present everywhere in the mixture in the size and with the electrical charge which they possess in the unmixed individual colloids *A* and *B*. Things are different, however, when small portions of *B* are added one after the other to *A*. Under these circumstances, if the reaction does not take place too rapidly, new aggregates of *B* and *A* are formed upon the addition of the first amount of colloid, which are not entirely neutral and which differ in size and charge from the original

particles of A. Every new addition of B will encounter new conditions in this regard, and, as experience has taught, usually conditions less favorable so far as precipitation is concerned. The result is that under these conditions of *partial saturation* more precipitating material is used up than when all is added at once, and that a series of intermediate bodies between the pure substances A and B and the fully neutralized mixture AB are formed. These intermediate bodies are built according to the type $xAyB$, in which x and y vary within certain limits.

A third possibility that interests us is the following. The aggregates formed through neutralization of the particles of two oppositely charged colloids are often held loosely together through slight electric forces. If an excess of one or the other colloid is added to such a precipitate, the new particles will, because of their electrical charge, enter into competition with the attraction forces existing in the neutral aggregates, and by diminishing their size and electrifying the particles cause the precipitate to go back into solution. As soon as a certain quantitative relation exists between the two colloids, the *precipitate will therefore attain a maximum* and will go back into solution as soon as one or the other colloid is present in excess. This is a familiar and well-studied relation existing between colloids.

The experimental facts that have just been recited were arranged so as to be ready for immediate application to one of the most important and interesting chapters of medicine, the immunity reactions. We shall deal more particularly with that difficult and much-argued relation between toxin and antitoxin. On thiss ubject

there exist a large number of valuable quantitative investigations, of which must be mentioned in particular the fundamental work of EHRLICH on diphtheria toxin.

I may perhaps be allowed, before entering into details, to touch upon those few leading points which render possible a simpler and more satisfactory conception of the relation of toxin to antitoxin than has until now been possible. Two assumptions suffice, both of which rest upon a fully established experimental basis and are generally accepted without contradiction: the colloidal constitution of toxins and antitoxins, and their ability to neutralize each other. Through these suppositions is rendered possible the extension to the dark relations existing between toxin and antitoxin of our advanced insight into the process of colloidal precipitation. As an actual matter of fact, we are dealing in both cases with a neutralization of colloids, only the criterion which exists to show that such a neutralization has occurred is different in the two cases. For in the first case this consists in the production of macroscopically visible aggregates, in the second in the formation of non-poisonous ones. The similarity between the general laws governing the two sets of phenomena is, in truth, striking.

It is a well-known fact, for example, that especially diphtheria toxin when kept for longer periods of time undergoes changes which are attributed to the formation of various complexes from the originally simple toxin. An analogous phenomenon is counted among the first-observed and best-known facts of colloids in general. It is dependent upon the presence of neutralizing impurities which are in the course of time able to render manifest their effect in the production of aggregates. The more

concentrated a colloidal solution, the richer it is, other things being equal, in impurities, and the less stabile in consequence. In the course of time the process of neutralization in the colloidal solution comes to an end, after which it remains stabile. EHRLICH has been able to observe similar facts in the case of diphtheria toxin which is kept a sufficiently long time. And we know from the observations of PALTAUF on the loss in strength of stored immune sera, and a recent excellent investigation of PICK and SCHWONER, that in antitoxin, especially in the case of the high-potency sera, the tendency to form aggregates is very great. That this tendency toward the formation of aggregates must be subject to the greatest variations under our present system of obtaining the impure toxin and antitoxin solutions is readily intelligible. In the preparation of inorganic colloids we have also only lately and by no means in all cases succeeded in obtaining stabile and uniform solutions, through perfection of technic and ideal cleanliness of material.

Upon the variations in the original properties of colloids are also dependent the variations in the colloidal mixtures. This explains that variability which has been observed in the behavior of toxin-antitoxin mixtures and which originally wrought great confusion in this question. We have already touched upon the great differences shown in the behavior of colloidal mixtures, depending upon whether they are precipitated at once or in fractions. It is of value to enter a little into the details of the relations existing when toxin and antitoxin are mixed together, for the observations of EHRLICH on the fractional saturation of toxin constitute the

foundation of our modern conceptions of its nature. If we add to a certain amount of a colloid A a much smaller amount of a neutralizing colloid B, then, generally speaking,* a part of A is not *completely* neutralized, while the remaining part remains free, but B is distributed over and combines with as large a number of the colloidal particles of A as is possible. As a result, new, incompletely neutralized aggregates are formed. By this means the number, size, and electrical condition of the particles become changed, and it depends entirely upon the character of these new values whether the tendency toward a formation of further aggregates upon the addition of a second portion of B is favored or inhibited. When such a second addition is made, new aggregates with new properties are again produced. If we have determined the amount of antitoxin necessary to just neutralize a lethal dose of toxin, we will need n times this amount to neutralize an n-times dose of toxin. Things are different, however, as soon as we try to saturate gradually, through the addition of succeeding small amounts of antitoxin, an n dose of toxin, as EHRLICH has done. Under these circumstances we can get only

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* It cannot be discussed in this paper in how far neutralization velocity and reversibility of the formation of aggregates determine the character of the course of the reaction. The more important instances can, however, be reviewed. Reversibility is always only partial, and decreases steadily from the moment of neutralization Even such stabile colloidal changes as the coagulation of egg albumin through heat or concentrated mineral acids are reversible at the moment that they are brought about. In the precipitation of protein through phenol, alcohol, or neutral salts the effect of time and the degree of reversibility increase from the first toward the last. This general property of colloids has in recent discussions of immunity assumed an important part under the heading of secondary fixation of toxin-antitoxin.

those transitional toxin-antitoxin compounds which show great variations in their reactions. This phenomenon has been responsible for the development of a rich nomenclature. All the relations between toxin and antitoxin are much complicated by the fact that diphtheria toxin, as has already been pointed out, may from the start enter into a reaction with antitoxin, with aggregates which are by no means all alike. To this is still to be added the following fundamental peculiarity in the reactions of the toxin. The toxicity of diphtheria toxin and its relations to antitoxin are able to vary independently of each other, a behavior which finds expression, for example, in the fact that a toxin requires approximately the same amount of antitoxin for neutralization no matter whether its toxicity is high or diminished through age. This phenomenon, which represents another of EHRLICH'S discoveries, was explained by assuming that the toxin is composed of a haptophore, or binding group, and a toxophore, or poison-bearing group. We must not fail to consider, however, that we are dealing with a reaction of the colloidal toxin with two different kinds of colloids —with the antitoxin and with the constituents of the cell.

Slight changes in the colloidal properties of a body are, however, able to affect one part of its reactions and leave another part free. An interesting example of this is furnished by the behavior of colloidal gold toward mercury, which I introduce because we are dealing under these circumstances with reactions between elements, reactions which no one will be inclined to attribute to properties of special atomic groups. Colloidal gold shows the properties of the pure metal, except that it

does not form an amalgam with mercury. A similar behavior can be mimicked in immunity reactions when the haptophore groups are lost while the ergophore groups are retained, or conversely. According to BILLITZER'S observations, it is the fact that both metals possess the same electrical charge which prevents the formation of an amalgam. How easily, however, it may be concluded from work with colloidal mixtures that new chemical compounds have been produced is indicated by the interesting fact that no less a man than BERZELIUS did this in a study of colloidal gold and came to similar conclusions, as did EHRLICH in a study of toxin-antitoxin mixtures. In gold-purple, which has been recognized through an excellent investigation of ZSIGMONDY as a mixture of colloidal gold and colloidal stannic acid, the gold shows some variations in reaction. Misled by this fact, BERZELIUS drew the conclusion that gold exists in gold-purple as the oxide. It is a fact of great importance that, in a mixture of two colloids *A* and *B*, the properties of *A* disappear for many reactions, while for others those of *B* disappear. According to the beautiful experiments of ZSIGMONDY, in a mixture of orthostannic and metastannic acids certain of the chemical properties of the metastannic acid are concealed by the ortho-compound, while toward other reagents the properties of the ortho-compounds fall entirely into the background. Every species of animal represents a different reagent toward the same mixture of toxin and antitoxin, in which at one time the effect of the toxine, at another that of the antitoxin, may hold the upper hand. Besides the phenomena of neutralization, this fact has done most to support the belief in the existence of toxins having different

chemical compositions. We are able to recognize in this only a general property of colloidal mixtures, the laws governing which ZSIGMONDY has formulated in his investigations on CASSIUS's gold-purple in the following way:

" As most important I consider the recognition of the fact that a mixture of colloids may, under certain circumstances, behave as a chemical compound, and that the properties of one of the constituents of such a mixture may be concealed through those of another."

An especially remarkable illustration of the identity of the neutralization of toxin with antitoxin and the neutralization of two colloids is furnished by a phenomenon which has also been discovered by EHRLICH, and a description of which we will introduce, with a few figures.

As the standard of a lethal dose of diphtheria toxin is taken the amount which will kill a guinea-pig of 250 grams in from four to five days. EHRLICH added to one immunity unit--that is, one cubic centimeter of antitoxin serum, capable of counteracting the poisonous effects of 100 simple lethal doses--enough diphtheria toxin until the mixture showed no toxic properties and indicated the amount of toxin necessary to accomplish this by L_0. This is usually less than one hundred, for the neutralizing toxin has, as a rule, stronger neutralizing than toxic properties. Without a knowledge of the properties of colloids one would expect that through the addition of a simple toxic dose to the approximately neutralized mixture L_0 one would obtain a product capable of killing a normal guinea-pig in from four to five days. EHRLICH found, however, that several simple toxic doses have to be added before the mixture again assumes the effect of a free lethal dose. From our knowledge of the formation

of aggregates in the process of colloidal precipitation, this result was to be expected, for each of the toxin doses added to L_0 distributes itself over all the aggregates already present, in consequence of which the mixture can attain the unit toxicity only after several doses have been added to it.

Through an investigation carried out by the chemist BILTZ, who has brought much light into this field, we are familiar with phenomena observed upon inorganic colloids that are entirely analogous to the EHRLICH phenomena just described. BILTZ studied quantitatively the neutralization of arsenious acid by its well-known antidote, iron hydroxide, of which the former represents an electronegative radicle, the latter an electropositive colloid. These investigations showed that a neutralized mixture of the two—that is, one corresponding with the L_0 toxin-antitoxin mixture, therefore—still has the power of uniting with arsenious acid and rendering several poisonous single doses harmless. A certain analogue of the EHRLICH phenomenon in the field of precipitation is perhaps to be found in the following. It is often possible, as has already been pointed out, to dissolve a precipitated colloid in an excess of the precipitating colloid. If the addition of a colloidal solution to a neutralized precipitated mixture corresponding with the L_0 value of EHRLICH is continued until the precipitate is redissolved, much more of this colloid is required for this purpose than when a colloid has added to it all at once an excess of a second colloid. The intimate connection between this phenomenon and the behavior of a colloid when precipitated through the addition of successive small doses of a second colloid can readily be seen. The procedure is the same,

only it is executed in different time and follows a different scale.

We still have to consider a few possibilities which can be deduced from the properties of differently charged colloids and which are realized in the phenomena of precipitation and in the phenomena of neutralizing a toxin. One of these is the antagonistic effects that small and large amounts of one colloid may have upon a second. Number, size, and charge of the particles of a colloid need not at all be related to each other in such a way as to best favor neutralization. For this reason different colloids are not precipitated with the same ease. Under certain circumstances a decrease in the electrical charge with a slight change in the size of the particles may make a colloid more stabile. This may be brought about through the addition of the right amount of a neutralizing colloid. Gelatine in small amounts may, for example, protect another colloid against a precipitation which at a greater concentration it itself brings about. A striking example, which until now has been regarded only as a curiosity, of such an antagonistic effect of one and the same colloid has been studied by JACOBY, who found that the toxicity of crotin is increased through the addition of small amounts of antitoxin, while it is decreased and neutralized through the addition of larger amounts.

A well-recognized conclusion to be drawn from the behavior of colloids toward each other is the following: It is by no means immaterial whether a colloid *A* has small amounts of a colloid *B* added to it, or whether *B* has small amounts of *A* added to it, and the aggregates formed in the two cases will, in general, be different

from each other. If we begin with an excess of toxin to which successive small amounts of antitoxin are added, the formation of aggregates will be able to follow a different course than when the reverse is the case, when we start with an excess of antitoxin. EHRLICH has worked according to the first method, while PICK and SCHWONER utilized the second method and came to the conclusion that the laws governing the combination between toxin and antitoxin were totally different from those discovered by EHRLICH.* It can easily be seen how through changes in only a few conditions an enormous variety in the character of the colloidal reactions is brought about, a behavior which is rendered apparent through a consideration of the numerous well-studied transitions existing between colloids and crystalloids. A colloidal solution consists of a suspension of fine particles which have assumed an electrical charge through giving off ions, just as have electrodes. If now the suspended particles become steadily smaller, while at the same time their electrical charge grows, they approximate more and more the behavior of ions, until finally the colloid passes over into a crystalloid, which dissociates

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* The relations existing here can be illustrated also by examples of protein precipitation. The precipitation of protein through the heavy metals is dependent upon the neutralization of the negative protein through the positive colloidal metallic hydroxide. The precipitates formed are, however, not soluble to the same extent in excesses of the individual colloids. The silver-protein precipitate is, for example, soluble in an excess of protein, but not in an excess of the silver salt. In the former case we have to do with the formation of variable aggregates' in the second with simple neutralization according to the manner observed by Pick on his toxostabile sera when large amounts of anti-toxin have toxin added to them.

in aqueous solution into its strongly charged ions. In this way mixtures of antagonistic colloids may approximate in their properties salts that have arisen from combinations between weak acids and weak bases. Strictly speaking, we are compelled to assume the existence of at least traces of such a similarity in order to account for the traces of the free substances which we find beside the aggregates in toxin-antitoxin mixtures. Some toxin-antitoxin mixtures might, finally, because of their close relationship to the crystalloid salts, contain the free substances beside completely neutralized aggregates. According to the investigations of ARRHENIUS and MADSEN, it is not impossible that such a state of affairs exists in the case of their tetanolysin. We are acquainted with an excellent experimental procedure for analyzing colloidal mixtures which BILLITZER has employed in a study of the relations existing in mixtures of the electropositive red iron hydroxide and the electronegative yellow arsenious sulphide. If an electric current is sent through such a mixture, the completely neutralized aggregates do not move, while the unneutralized aggregates, which carry the electric charge of the colloid that is present in excess, are slowly carried to the oppositely charged pole. The traces of free colloid still present move most rapidly and to opposite poles, where they evidence themselves in this case through differences in color.

The transition from the complicated conditions existing in the case of the toxins to the more simple ones in the case of the precipitating and agglutinating substances is rendered easy through the fact that in the latter case we are dealing with the reactions of the relatively well-understood proteins, or substances closely

related to them. With this we come to the question of the colloidal properties of the proteins.

If an electric current is carefully sent through a solution of egg albumin poor in salts, the protein migrates, as shown by chemical analysis, to the positive pole. This migration is very slight, and, since the protein particles have been by optical means proved to be very small, must be attributed to a weak electronegative charge which they carry. A current of 250 volts for twenty-four hours is required to render evident this migration of the protein. The slight charge and the minuteness of the particles explain the very considerable stability of the protein toward precipitating ions. While the more strongly charged colloidal metals and the majority of the inorganic colloids are precipitated in weak salt solutions, this is not true of protein. Through this fact is rendered possible the vitally important existence of salts and protein side by side. If the protein particles are given a greater charge than they possess normally, they are readily precipitable. In the presence of acids, for example, the proteins assume a strong electropositive charge, as evidenced by their very considerable migration toward the negative pole, and are now readily precipitable through electronegative colloids. We make clinical use of this procedure daily when we first give dilute protein solutions a positive charge through the addition of acetic acid, after which, upon the addition of potassium ferrocyanide, they produce the well-known precipitate with the colloidal negative ferrocyanic acid. In fact, in the majority of the sensitive reactions for albumin, we have to do with the effect of an oppositely charged colloid upon a suitably electrified albumin.

The phenomena observed in precipitin and agglutinin reactions are explained in a similar way. These precipitations are possible only in the presence of salts. If the protein or the bacteria under investigation are mixed with the specific substances in a salt-free condition, no reaction occurs. These specific substances may, therefore, be looked upon as giving the colloidal proteins the properties of sensitive colloids, that of being precipitated through small amounts of salt ions. According to BILLITZER the specific substances serve in this case only to give the colloidal particles the charge and size necessary for precipitation. Apparently all "sensitizing" reactions encountered in the realm of the immune-body reactions are explainable in a similar way.

A phenomenon frequently observed is furnished by the above-mentioned tendency of colloids to show an optimum proportion in which the two reacting colloids must be mixed in order that they may be precipitated, and by the inhibition of the reaction when an excess of the one is present. BILTZ has already pointed out the existence of this generalized phenomenon of colloids in agglutination; analogous phenomena may, however, be observed in nearly all immune-body reactions. A remarkable example of this kind is furnished by the "complement diversion" (*Komplementablenkung*) observed by NEISSER and WECHSBERG. These authors showed that bactericidal immune sera showed a maximum effect, under otherwise similar conditions, when they contained a medium amount of immune substance. No doubt we can with' profit now express the description of this phenomenon in the smoother language of colloid chemistry. The complex relations existing in the case of hæmolysis, which

may be brought about in a great variety of ways, also seem clearer as soon as the exit of the coloring-matter from the blood-corpuscles is looked upon as a rupture of the colloidal hæmoglobin-stroma compound. There seem to exist, therefore, a large number of light-bringing relations between immunity phenomena and phenomena in colloid chemistry. LANDSTEINER in conjunction with JAGIC have been able to show the identity existing between the mechanism of the hæmolysis (studied by KYES and SACHS) brought about through the unknown cobra poison and the mechanism of the hæmolytic effect of colloidal silicic acid. In this case a structurally uniform inorganic colloid behaves like the hæmolytic amboceptor of the side chain theory.

We are indebted to the same investigator for recognizing a fact of still more general significance. The proteins, and from many facts at our disposal the immune bodies also, represent so-called amphoteric electrolytes; in other words, substances which assume basic properties in acid solutions and acid properties in alkaline solutions; or, as shown by experiment, change the sign of their electric charge with a change in reaction. There exists, however, a zone between the extreme changes in the sign of the electrical charge in which these hermaphrodite-like substances respond to the slightest change in their surroundings with an alteration in their electrical character, through which the existence of a large number of finely graded relations between amphoteric electrolytes differing only slightly from each other is rendered possible. This enormously changeable sensitiveness of such substances, which may, according to circumstances, act at one time as colloids having one kind of electrical

charge, at another time an opposite charge, is evidenced by a large number of facts. With this conception of the rôle of amphoteric substances, LANDSTEINER has made the first rational attempt to explain the specificity of the immune substances.

The chemistry of the colloids also allows us to assume a freer position regarding the hypotheses governing investigations in immunity. We can, however, touch upon this subject only briefly here, and must limit ourselves to the question of toxins and antitoxins.

Every one is familiar with the dominating influence which those views have at present attained that EHRLICH has developed under the name of the side chain theory, views which have not, of course, remained without contradiction. The opposition which this theory has encountered has evidenced itself silently in the fact that a number of investigators have continued to work independently and without using it, and audibly through the expressions of various authors, more particularly MAX GRUBER, who delivered an address in Vienna several years ago.

As already stated, EHRLICH has, among others, utilized the fact that the affinity of toxin for antitoxin and the toxicity of toxin may vary independently of each other, to support the idea that two different groups, a haptophore and a toxophore, exist in a toxin. These are supposed to furnish the material substrate for the different reactions of which one and the same substance is capable. EHRLICH has at the same time distinguished between different varieties of a toxin, originating in part from the bacteria themselves, such as the toxones, and in part the

product of time when the toxin is kept for a long time, such as the toxoids. The differences observed in the toxicities of a toxin when only partially saturated with antitoxin, and those observed in the behavior of different animals toward the neutralized toxins, all furnish important support for these assumptions. But we must look upon it as a fact well established through investigations on colloids, that in their changes in state a part of their reactions may be influenced, while another part may remain untouched; that through the mixture of colloids which neutralize each other manifold new, in no sense preformed, aggregates can be produced, and that such aggregates may at one time allow the effect of the one colloid,—in this case the toxin,—at another time the other, —the antitoxin,—to become apparent. Great differences must in consequence result in the intensity and in the picture of the intoxication in different animals. Against EHRLICH's theory ARRHENIUS and MADSEN, as well as GRUBER and PIRQUET, have set up the idea that in toxin-antitoxin mixtures we always have to do with a dissociation of compounds having only a weak affinity for each other. This conception, which originated from a too far-reaching generalization of a special case, allows only of the existence of completely neutralized aggregates, beside traces of their components, and is, therefore, incapable of explaining the multitude of experimental facts which have been obtained in the study of different toxins, more especially diphtheria toxin. Against this inadequate assumption arose the strength of the EHRLICH theory, which has rendered the great service of having been the first to fix in the minds of investigators the newly discovered and scarcely calculable varieties of

facts won through a study of immunity. But the facts of colloidal chemistry, together with advances in the investigation of immunity, show clearly, it seems to me, that the EHRLICH assumptions, in spite of the many variables introduced into them, do not at all suffice to explain the phenomena actually observed.

It is, for example, an easy matter to foretell even now that through changes in the manner and the rapidity with which toxin and antitoxin are mixed, and through a proper choice of animals, the varieties of toxins that have been assumed to exist by EHRLICH might easily be increased indefinitely.

It can be readily seen, too, how difficulties arise in the expansion of any theory that is based upon crystalloidal chemistry. With EHRLICH it is the application of synthetic organic chemistry, with his opponents, the application of a special case of the dissociation of salts that finally constitutes too narrow a frame to receive the entire picture of facts. Insufficient also was the attempt of DANYSZ and BORDET to explain the behavior of toxins. The latter especially tried in his brilliant way to support his theory through an analogy with the process of dyeing, which we now know to be a colloidal reaction. In this theory the correct assumption that toxin and antitoxin are able to unite in different proportions was made; it could, however, be of value only as a hypothetical objection, as it included only some of the possibilities and was unable to explain, while lacking the broad base of other facts in colloidal chemistry, the variety of observations made on toxins. If we disregard my first brief suggestion, pointing out the relation between immunity reactions and colloidal changes in state, LAND-

STEINER was the first who independently, and recognizing the goal toward which he was travelling, studied this connection between immunity and colloidal reactions experimentally. LANDSTEINER has done this more especially for agglutination and hæmolysis, while I have attempted to do it for the true toxins and their antitoxins.*

The extensive importance of colloidal chemistry for biology is by no means limited to the highly interesting field of immunity. This can be shown to be true to-day, however, on only a few examples.

We are acquainted with a remarkable kind of separation of solid colloids through the action of surface tension, an understanding of which is of importance in many problems of pathology.

The nature of these forces which evidence themselves

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* I am, of course, aware that valuable beginnings have already been made to apply the facts of colloidal chemistry to the teachings of toxins and antitoxins, and nothing is further from my mind than to disregard the great credit which more especially BILTZ deserves in this respect. I would, nevertheless, like to emphasize that my conceptions are independent ones which developed gradually—as such things must—in the course of my investigations of organic colloids. They form only a special case of the analogy which I have for more than a decade tried to show exists in the most varied subjects between changes in the state of colloids and the processes that go on in living matter. My treatment of the subject is, moreover, directed toward numerous until now scarcely prized, but apparently most important, sections of the problem.

For the parallelism between the precipitation of a colloid and the neutralization of a toxin by an antitoxin which I have given above, the experiments of COEHN, according to whom toxin and antitoxin wander toward the same side in the electric current, are of no importance, as I have been able to show in my unequivocal investigations.

at the surfaces of all liquids is familiar to every one. While the particles within the liquid are surrounded on all sides by particles of the same kind and are in consequence in a state of equilibrium through the uniform distribution of the attractive forces about them, this is not the case with the surface particles. For here the forces which act upon the particles and are directed toward the centre of the liquid encounter no corresponding antagonistic force. In consequence of the effort of the surface particles to follow the attraction toward the centre, the surface endeavors to become as small as conditions will permit. Many substances are able through solution in a solvent to bring about a decrease in its surface tension. Under these circumstances the liquid may follow its tendency to decrease its surface as much as possible by allowing the particles of the substance which decrease the surface tension to take the place of the particles of the liquid which are being pulled toward the centre. In this way the surface becomes gradually richer in the dissolved substance. In the end the concentration of the solid particles at the surface becomes so great that a film is formed which may be removed and which is renewed after each removal. This behavior, which has been studied in great detail by RAMSDEN, is shown chiefly by the colloids, more especially protein solutions. If such solutions are shaken together with air or immiscible liquids, such as oil, chloroform, or mercury, all the dissolved colloid can finally be precipitated through the surfaces which are constantly renewed.

That this process should be demonstrable most clearly on colloids and especially on protein solutions is due to

two still other conditions, according to our judgment. If the coagulation through surface tension is studied in detail we find that we have to do with an extreme increase in the concentration of a solution, or, what amounts to the same thing, with the expression of the solvent from such a solution. The energy necessary for this, which, as is well known, expresses itself in osmotic pressure, is, however, in the case of the colloidal substances, exceedingly small in contrast to that observed in crystalloidal substances. And so it is that the colloids are especially well able to form solid surface films quickly and extensively.

A second important condition is the electrical charge of the colloidal particles. The greater this is, the more powerful are the repellent forces between the similarly charged particles that antagonize the surface tension, which tends to crowd them together. The proteins, as we know, carry only weak charges of electricity and furnish in consequence favorable conditions for the collection of the particles on free surfaces. To the formation of such solid protein films RAMSDEN attributes the well-known formation of a film on milk and the behavior of the fat droplets of milk, which have long been imagined, from their physical and chemical reactions, to be surrounded by a denser film of protein. In pathology similar phenomena might play an accessory part in air and fat embolism. It can readily be shown experimentally that the migration of air bubbles in tubes which are filled with protein solutions meets with unexpectedly great difficulties. In the living organism the danger of the entrance of air bubbles or fat droplets into the circulation is dependent, at least in part, upon similar processes.

In both cases we have to do with a solidification of their surfaces which makes the emboli behave, in spite of their gaseous or liquid character, like solid obstructions to the circulation.

In a new light appear also, through a study of the colloids, the extracellular phenomena observed in the development of solid supporting substances, such as cartilage and bone, and the precipitation of crystalloidal substances in the tissues connected with them. The latter question, which is also of great pathological importance, has aroused much interest in the past decade, but it does not seem to have been possible to get far beyond the recognition of the difficulties which the solution of the problem encounters. The nucleus of the question seems to lie in the fact that we are dealing, on the one hand, with simple processes of crystallization, while, on the other hand, formative influences on the part of the cells and functional adaptations to the forces destined to act upon them seem to be clearly discernible in the arrangement of the crystallization. Besides the studies of numerous investigators on the process of ossification, it has been BIEDERMANN, more especially within recent years, who, through his excellent work on the shells of molluscs, crustaceans, and insects, has furnished much important experimental material.

The importance of colloidal chemistry in these problems evidences itself at once in that first and most important question of the conditions under which the scarcely soluble salts are kept in solution and precipitated in suitable places. The colloids constitute, as shown by many experiments, an excellent means under certain circumstances of keeping slightly soluble salts in solution,

in that they themselves unite with the salt ions and form aggregates without necessarily being precipitated, when the size and charge of the particles, as in the case of protein, are small. It is, for example, an easy matter to show that by no means inconsiderable amounts of barium sulphate, one of the least soluble of substances, can be kept in solution in serum. If now, through the metabolism of the cells, the dissolving colloids are destroyed and not replaced, supersaturation and finally precipitation of the crystalloidal material can easily be brought about, after which, as is so often the case, crystals that have once been formed may serve as centres for further crystallization. This conception seems to make clear the connection between crystallization and the vital activities of the cells, as evidenced by the organization of the supporting tissues. It renders intelligible also the regularly observed phenomenon that a small portion of colloidal material, which is no longer able alone to keep the salts in solution, always crystallizes out with them. In this same direction is to be sought the connection between pathological processes of calcification, deposition of uric acid, and tissue metabolism. If the beautiful investigations of PAUL and HIS on the conditions determining the solubility of uric acid are not of that importance for the pathogenesis of gout which they had hoped, then this is dependent primarily upon the fact that the same conditions of equilibrium which exist in the organism in the presence of colloids were not established in their experiment.

A special problem is presented by the origin of the great solidity of cartilage and bone, or rather their peculiar colloidal intercellular substance. These tissues

possess a characteristic, highly concentrated ground sub-
stance, connected with structures which represent, in
the main, thin layers deposited about the formative cells
or their delicate protoplasmic extensions. A very in-
structive example of this kind is furnished by the exceed-
ingly hard cartilage of myxina, in which SCHAFFER was
recently able to demonstrate layers of great delicacy sur-
rounding the cartilage cells. In such thin precipitation
layers there can arise, when they absorb or gradually
give off water, tremendous pressures which might well be
of great importance in hardening the entire mass. Ac-
cording to experiments which I have carried out in conjunc-
tion with Dr. LUDWIG MACH, it is an easy matter to give
protein a bony hardness by pressing it into a steel tube
and heating it almost to its decomposition temperature.
Interestingly enough, this ability to form a material
sufficiently hard to be worked with instruments is con-
nected with a certain integrity of the albuminous sub-
stance and is lost entirely when the albumin is decomposed
beyond the albumose stage. By mixing with the protein
the fine dust of insoluble calcium salts in the proportion
in which these are found in bone, the solidity of the prod-
uct can be markedly increased.

The pressures exerted by thin layers of colloid under
suitable circumstances cannot well be determined. That
these may attain high value is shown by experiments of
CAILLETET, who was able to render glass permanently
doubly refracting through thin layers of gelatine which
were allowed to dry upon its surface.

With this example, to which many others might be
added, illustrating the connection between physico-
chemical investigations and morphology, I shall close

what I intended to offer in the line of experimental facts.

If I have succeeded, as I hope, in convincing you also that the application of colloidal chemistry to physiology and pathology justifies great expectations, it might be well, in conclusion, to seek in the development and the present state of the colloid problem a measure for our faith in its future contributions.

As is well known, the difference between crystalloids and colloids appeared to be so radical a one to GRAHAM that to characterize it he wrote the following oft-quoted sentence:

" The difference between these two kinds of matter is like that which exists between the material found in a mineral and that found in an organized mass."

The discoverer of the colloidal world has since been reproached, and certainly unjustly, for having so strongly emphasized the differences between crystalloids and colloids. For every discovery depends primarily upon a recognition of the most apparent differences between the new phenomenon and the facts well known at the time. This contrast is the most powerful stimulus to investigation. It is the source of the problem, which is not solved until the apparent contradictions to it have been set aside and those fine threads have been unravelled which connect the newly found with the old. Our problem also developed in this way, and we have seen how the connection between colloids and crystalloids was established through a recognition of their principal characteristics and their gradations. One is almost inclined to believe that the possibility of explaining

organized living matter through his colloidal condition seemed close at hand even to GRAHAM.

The continued application of colloidal chemistry to biological problems soon shows, however, the limits which are set upon it. We are able to see this in the case of the antibodies also. How much a knowledge of the colloids contributes toward an understanding of their varied reactions and even the solution of the riddle of their specific sensitiveness toward each other seems most apparent. Our new methods are of no use, however, as soon as we try to discover the secret mechanism by means of which the cells produce a suitable antitoxin against any definite toxin. To repeat the words of GRUBER: "Whence comes this astonishing purposefulness, this predetermined harmony, this specific adaptation of substance to antisubstance, which one would a priori consider entirely impossible?"

It must seem remarkable that, instead of exhausting one's self in chemical analogies, one has not sought a more intimate connection * with those phenomena which arise from a direct observation of living matter. Beginning with his classical investigations of the physiology of the senses, EWALD HERING has developed a theory of the changes that go on in living matter, which through abundant use of the principle of mobile equilibrium has foreshadowed modern chemical dynamics. We need only to recall how, according to HERING, the sensations of antagonistic colors correspond with antagonistic reactions in the visual substance which mutually suppress each other. When we consider that the substances of the

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* Only LANDSTEINER makes a similar suggestion.

red process produce in their surroundings or after them the antibodies of the green process,* and that these products neutralize each other physiologically, just as do toxin and antitoxin, the analogy between the two phenomena becomes very apparent.

In spite of a few differences, are not the chemical phenomena of the production of a lasting complementary after-image and the formation of an antitoxin essentially the same? Even in EHRLICH's bold conception of the regenerative hyperplasia of the side chains, there seems to be mirrored only a part of that truth which HERING grasped so deeply.

No doubt every attempt to follow these questions further soon brings us to the solid barriers of our present knowledge, which do not open even to the storm of physical chemistry. And if some investigators, such as the followers of the energetic school, carried away by physical chemistry, have believed that they had hoisted their flag upon the outermost pole of biology, it has always been found that this was due to a failure to discriminate between the boundaries of the known and the unknown. We have therefore learned to be satisfied with having arrived at a clear conception of the problem before us, just as the chemist who must first carefully free an unknown substance of all its impurities before he holds the pure crystal in his hands. As yet the way

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* For comparison the white-black process might better be used, because this leads to black in only one direction, namely, over white to black. In the case of the antibodies also we obtain the antibody only by way of the toxin and not conversely. The exceptions mentioned in the sentence following the one to which this note refers have to do with another point which will be discussed in detail at some future time.

is not apparent along which it will one day become possible to discover its structure.

7. On the Electrical Charge of Protein and its Significance.*

I.

THE surprising development of the chemistry of the colloids, which in no small part has been incited through its great biological importance, has reacted most beneficently upon the latter science and many problems in general physiology. I have repeatedly had the honor of bringing before this society reports of that daily increasing territory in which the study of colloidal reactions touches upon or coincides with that of the structure and changes in state, and in consequence the functions of the cells and fluids of the organism.

If one attempts to survey the long series of colloidal substances and to study along the lines common to all, it must become apparent to every one how markedly their typical properties vary in degree in spite of a certain identity in behavior in the matter of diffusion, for example; and how the presence or entire absence of certain properties changes the whole character of a colloidal reaction. This holds not only for the fundamental differences between the solid and jelly-like colloids, or gels, and the liquid colloids, or sols, but also for the individual members of each of these groups. In fact, we see that among the sols the proteins constitute an almost independent

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group because of numerous properties that they have in common.   This behavior will, no doubt, have to be taken into consideration by any one who attempts to extend the analogy of the behavior of colloids in general to that of the colloidal substances in the fluids and tissues of the organism.   For it has been found that the reactions of all colloids do not approximate the reactions that occur in the living body, in consequence of which it has proved necessary, in the attempt to discover such analogies, to cling to the colloidal products of living matter itself, namely, to the proteins.

The value of an accurate knowledge of the proteins as a means of understanding the inner workings of life phenomena has at different times been differently estimated by physiologists.   While a time once existed when many believed that a knowledge of protein structure would by itself give us an explanation of the peculiar metabolism of living matter, we have to-day, when the beginnings of a protein synthesis are apparent and many important constituents of the protein molecule have been isolated, become quieter and soberer in our expectations. Largely independent of a complete insight into the chemical composition of the proteins is the knowledge of their physico-chemical properties, which can only be obtained through utilization of different methods.   This knowledge gives us an immediate understanding of the majority of the general properties and functions of the tissue fluids, and is applicable without reserve to those cases also in which no longer living but more or less coagulated cell material serves as an object of research. In the end, however, such a knowledge also renders easy an insight into the changes that take place in living

cells in consequence of the frequently recognizable parallelism between changes in state in colloids and changes in physiological function. This is no doubt dependent upon the fact that the colloidal constituents of living matter show, at least in part, a physico-chemical identity with the properties of isolated proteins.

It is our purpose to-day to give as far as possible a survey, based on the personal investigations of many years, of the more important physico-chemical properties of the proteins, and to point out, at least in a cursory way, the relation between these properties and many biological phenomena.

II.

As in the case of crystalloids, so both the behavior in solution and the behavior in the solid precipitate serve to characterize the colloids. An accurate knowledge of the conditions which determine their precipitation has recently assumed great importance in the study of the colloids.

We can to-day regard it as settled that between a true suspension and a colloidal solution there exists only a difference in the size of the suspended particles. In a colloidal solution they are always so small that through their friction upon each other they are kept in suspension. The colloidal particles seem, therefore, to be no longer affected by the force of gravity, just as is the case with those smallest dust particles in the air that become visible only in the sunlight. The colloidal particles can also be rendered visible in many cases by utilizing intense illumination methods. Even though gravity is unable to cause a clumping of the colloidal particles, other forces

are readily able to do so, especially electrostatic **forces,** with which we are going to deal chiefly to-day.

As is well known, many crystalloids, such as salts, **acids,** and bases, give off their constituents at the electrodes when a current is sent through them. We call such substances electrolytes, and a much-used theory assumes, as is well known, that there exist in aqueous solutions of electrolytes, besides the electrically neutral molecules, the electrically charged dissociation products, the so-called ions. Upon the migration of these ions toward the electrodes is dependent the conduction of electricity. The ions are said to be positive when they wander to the negative pole to be discharged and deposited, and negative when they wander to the positive pole. In this way the H ion, which all acids have in common, is electropositive, the remaining portion of the molecule electronegative, In the same way the OH ion, which all alkalies have in common, is electronegative. The strength of an acid or a base is determined by the concentration of these ions.

Colloids behave in an entirely different way. If an electric current is sent through a pure colloidal solution, the colloidal particles move, in contrast to the electrolytes, in only one direction. They accumulate at either the positive or negative electrode, from which we conclude that they have either a negative or a positive charge. A connection has shown itself to exist between this electrical charge and the process of precipitation. Several investigators, more especially BILTZ, have shown that only oppositely charged colloids mutually precipitate each other, and that the entirely precipitated colloidal material no longer has an electrical charge, that is to say, no

longer moves with the electric current. Even before BILTZ's work other observations had indicated the importance of electrical conditions for colloidal precipitations and had formed the starting-point of theoretical explanations. The colloids seem, in general, to be precipitable only through electrolytes; non-electrolytes such as sugar or urea have no precipitating effect even upon very unstabile colloids. HARDY and BREDIG have, in accord with the theory of electrocapillary phenomena, developed the idea that there exists an antagonism between the forces of surface tension, which, according to BREDIG, cause the colloidal particles to coalesce, and the electrical charges that the colloidal particles carry, in such a way that only after the electrical charge which causes the particles to repel each other has been removed can the surface tension attain its maximum. If a discharge of the colloidal particles is brought about through the addition of the oppositely charged ions of electrolytes, then the optimum of precipitability is produced at the same time, and a precipitate is formed.

According to a different theory developed by BILLITZER, surface tension does not play the rôle attributed to it by HARDY and BREDIG. If oppositely charged ions are added to a colloid, the colloidal particles collect about these ions through electrostatic attraction. In this way aggregates are finally formed of sufficient size to fall to the bottom. According to this view, with which many facts agree that contradict the first-mentioned theory, a colloid carrying no electrical charge should be capable of precipitation only with difficulty, as its particles exert no electrostatic forces. One can regard these theories as one pleases; no doubt the necessity of testing the

electrical behavior of dissolved protein in order to obtain a better insight into its colloidal reactions will be apparent to every one. I planned, therefore, to obtain, first of all, native protein as free from electrolytes as possible in order to have a stock material for testing the effect of different conditions upon the electrical behavior of protein. From the standpoint of general technic, moreover, it seemed of great value to use a material which through extreme dialysis, or this in combination with repeated freezing, had been rendered as free from salts as possible. The electrical conductivity rendered possible through the presence of ions is very great when compared with that produced through the migration of colloids. In order to render the latter apparent, very strong currents must therefore be used, which in the presence of salts lead to a great heating of the solution and also to a masking of the phenomenon sought for through the action of the products of electrolysis. In our experiments we used, for example, a current of 250 volts and 6 amperes. In this current ordinary blood serum burns, while our salt-free serum allowed only a few millionths of the current to pass through it. To test the migration of the colloid in the electrical current we utilized an apparatus similar to the one successfully used by BILLITZER in his beautiful experiments. Three beakers of uniform size were connected with each other by means of siphons. The electrodes dipped into the two outer beakers, while the middle one served as a control, the contents of which should, of course, not vary. At the conclusion of the experiment the nitrogen in all three of the vessels was determined by KJELDAHL's method.

The results of a long series of electrical convection

tests, in which the effect of concentration and other conditions was also determined quantitatively, may be thus summarized:

1. A protein which has been carefully freed from electrolytes shows no recognizable electrical charge and does not wander toward one of the two electrodes, even when subjected to an electric current for twenty-four hours.

2. Each of the albuminous constituents of the serum—serum albumin, pseudoglobulin, euglobulin—shows no electrical charge in the absence of electrolytes.

3. The addition of neutral salts of the alkalies or the alkaline earths does not impart an electrical charge to the uncharged protein.

4. Traces of acids impart a positive charge to protein through their positively charged hydrogen ions; alkalies a negative charge through their hydroxyl ions.

5. Salts with an alkaline reaction toward litmus, such as carbonates and the secondary and tertiary phosphates of the alkali metals, render protein electronegative; acid salts give it a positive charge.

6. This charge is independent of the end reaction of the medium. A proper mixture of protein and sodium bicarbonate is faintly acid toward phenolphthalein, neutral toward litmus; the protein has, however, a strongly negative charge.

III.

Let us consider, first of all, the fact that our salt-free protein carries no electrical charge. How does it behave toward the salts of the heavy metals, such as Cu, Fe, Zn, Pb, Hg, which are all regarded as general precipitants of

protein in even very dilute solutions?   All these salts are characterized by the fact that they undergo great hydrolytic dissociation in dilute solution—in other words, take up water and break up into their metallic hydroxide and their acid.   According to different investigations which agree in their conclusions, the dissolved colloidal electropositive metallic hydroxide is the real protein-precipitating constituent.   If now it is true that colloids mutually precipitate each other only through the opposite electrical charges which they carry, then the uncharged protein should in general not be precipitable through electropositive heavy metals.   It can be easily shown that our uncharged protein, in contrast to native protein, cannot be precipitated through salts of Fe, Cu, Hg, Pb, and Zn. This experiment harmonizes, therefore, with BILLITZER'S theory, according to which protein is very stabile in the uncharged state.

Let us now turn to something else.   As is well known, alcohol is an excellent precipitant for proteins.   Since alcohol as a non-electrolyte furnishes practically no ions in aqueous solution, its precipitating power cannot rest upon electrical grounds.   The matter may be explained in the following way: Proteins are not soluble in alcohol, but they are readily miscible with water.   The proteins are therefore crowded out of their solvent through the addition of much alcohol, in the course of which their small particles, by virtue of their surface tension, coalesce into larger aggregates in a way similar to the clumping of the particles of a fresh, fine precipitate into larger masses with time.   We will therefore not be surprised to see that our uncharged protein is readily precipitated by alcohol.   But what will happen if we first give this protein

a positive or a negative charge? We create in this way repellent electrical forces between the smallest particles of colloidal material, which will work against the surface tension, which tends to make them coalesce and precipitate. If the protein is given an electrical charge through the addition of a little acid or alkali, then, as experiment shows, its precipitation through alcohol is inhibited or entirely prevented. We may imagine from this that for precipitation through non-electric forces conditions must exist somewhat similar to those which HARDY and BREDIG believed to exist for electrolytes.

In passing it may be mentioned that our uncharged protein is readily coagulable through heat and, as may be imagined, through acetic acid-potassium ferrocyanide, phosphotungstic acid, and phosphomolybdic acid. In the first case we are dealing with an as yet not entirely understood chemical change in the protein brought about through the high temperature. In the second case the protein is first charged positively through the acetic acid, to be precipitated later by the various oppositely charged, probably colloidal, acid ions.

These conversion and precipitation experiments are able to answer the question, In what electrical condition do the proteins exist in the blood and the tissue fluids? Since alkalies impart a negative, acids a strongly positive, reaction to proteins, one is able to draw conclusions from the reactions of animal fluids as to the charge of the proteins contained in them. Modern investigations have solved for us the question of the reaction of the tissue fluids, or, to put it more accurately, the relation between their content of H and OH ions. According to these investigations, the body fluids are neutral. The free

hydrogen and hydroxyl ions exist in them in the same proportion as in water. This is shown most harmoniously not only through tests with proper indicators, such as phenolphthalein, but also through electrical measurements. Litmus, which was formerly employed as an indicator, is itself too strong an acid to show the presence of the weak acids of the tissue fluids, and indicates therefore an alkaline color reaction. If we remember that uncharged protein cannot be precipitated through the positively charged heavy metals, while the proteins of the tissue fluids can at once be precipitated by them, the conclusion is inevitable that native protein carries a negative charge. This charge can be derived only from the hydroxyl ions that are split off from the salts of the serum, which, in harmony with the above-described experiments, must be the carbonates and phosphates. If sodium bicarbonate is added to fresh non-charged protein, this assumes a strong negative charge even though the resulting mixture is neutral toward litmus and acid toward phenolphthalein. In an experiment conducted with such a sodium bicarbonate-protein, it was found that the relation of nitrogen at the cathode was to that at the anode as 3 : 5; while the nitrogen content of the middle beaker was expressed by 4.

IV.

We are now acquainted with sufficient facts to study more closely the conditions for the precipitation of native electronegative protein, and to compare these whenever necessary with those of uncharged or artificially charged protein.

Let us consider, first of all, the precipitation of native prote'n through neutral salts of the alkali metals and see in how far the ions play an immediate rôle. The following table, in which + indicates that the protein is precipitated, − that it is not precipitated, gives a good survey of these relations.

In the vertical row of the table are arranged the positive ions in the order of their decreasing power to precipitate protein; in the horizontal row are arranged the negative ions in the order in which they inhibit the precipitation.

→ Increase in inhibition.

↓ Decrease in precipitating power.		SO_4	$C_2H_3O_2$	Cl	NO_3	Br	I	CNS
	Li	+		+	+	+		
	Na.........	+	+	+	+	−	−	−
	K..........	+	+	+	−	−	−	−
	NH_4.	+	−	−	−	−	−	−

The antagonism between cation and anion is indicated by the appearance of one and the same ion in salts which precipitate and those which do not precipitate protein— for example, sodium as the sulphate and as the bromide; and if we go along still further in the sodium series the inhibiting effects of iodide and sulphocyanate ions have so much the upper hand that the presence of these salts prevents the precipitation of protein through other salts. As an actual matter of fact, the inhibiting salts were discovered as a consequence of the assumption of antagonistic ion effects. That it is the positive metallic ions which are the bearers of the precipitating power may be concluded from the fact that native protein carries a negative charge.

Let us now ask what will happen when we try to precipitate with neutral salts protein that has been rendered electropositive through the addition of an acid.   Theoretically we would expect that under these circumstances the negative ions of the salts would precipitate the protein, while the metallic ions would have an inhibiting effect. And as an actual matter of fact, we find that when the protein has been acidulated the formerly inhibiting bromides, iodides, and sulphocyanates become powerful precipitants, and those salts which formerly precipitated now inhibit.   In other words, the signs of the above table are reversed; only the precipitating effect of the negative ions increases in the same order as their inhibiting effect did formerly, while the order of the now inhibiting positive ions is just the reverse of that given in the table.

The following interesting fact has also been found. The same reversal in ionic effects as is brought about through acids can also be brought about through the addition of the salts of the alkaline earths, Ca, Ba, and Sr, to the native protein.   One may conclude from this that a change in the sign of the charge of the protein solution from the negative to the positive occurs in this case also.   According to our conversion experiments, however, uncharged protein does not assume an electrical charge through the presence of the neutral salts of the alkaline earths, and in consequence we have to discover whether these do not bring about an acid reaction by meeting with the salts contained in the animal fluids. In order to answer this question, let us consider the changes that are brought about through the addition of calcium chloride to a solution of sodium bicarbonate or disodium phosphate.   As is well known, the two salts

last mentioned have an alkaline reaction in that upon solution in water they increase the number of OH ions present in it. This is brought about through the fact that they combine with water and split hydrolytically into sodium hydroxide and carbonic and phosphoric acids. Since, however, sodium hydroxide is a stronger base than carbonic and phosphoric acids are acids, more OH ions exist in solution than H ions. If now we imagine the sodium hydroxide to be replaced by the much weaker calcium hydroxide, then the concentration of the free OH ions will fall immediately. The chemical reaction between the alkaline earths added to an animal fluid and the phosphates and carbonates contained in it must also act in this way—in other words, toward the establishment of an acid reaction in the fluid. It can readily be shown even in an experiment with native protein to which an alkali has been added and which reddens phenolphthalein very strongly that an acid reaction is produced in the mixture as soon as calcium chloride is added to it, as indicated by disappearance of the red color. In this way the identical effects of acids and alkaline earths upon negatively charged protein have found a ready explanation.

That the alkaline earths can act only indirectly through their effects upon the salts of the serum is shown most strikingly by an experiment in which calcium chloride is added to salt-free serum. If sodium iodide or sodium sulphocyanate is added to this mixture, no precipitate is produced. If, however, the protein is rendered electropositive through the addition of a little acid, then the sulphocyanate at once brings about a coarsely flocculent precipitate.

We will end with this our discussion of observations which indicate unequivocally the great importance of the electrical condition of the proteins for their reactions. Since this electrical condition of the proteins is determined solely through the non-neutral salts of the tissue fluids, we can readily see how important a proper balance of these salts must be for the organism. One will not err, therefore, in discovering, in the purposeful arrangements existing in the animal body against the presence of too large amounts of acid, instruments of protection for the proper physiological electrical charge of the proteins.

V.

We are, no doubt, justified in presupposing that conditions within the cell are very analogous to those found in the tissue fluids. Höber, for example, has found in an excellently arranged experiment that the red blood-corpuscles move to the anode—in other words, are negatively charged under normal circumstances and retain this charge under a great variety of conditions. If they possess in this wise an electrical charge which is similar to that of the blood serum, they can nevertheless show variations in their behavior, as, for example, under the influence of acids. In an isotonic cane sugar-sodium chloride mixture they become electropositive under the influence of carbonic acid, a change that is again reversed when the carbonic acid is removed. It seems, therefore, as though the red blood-corpuscles suffer a complete change in electrical reaction when they pass through the pulmonary circuit.

The essence of the electrical condition of cells can be

demonstrated without difficulty on the electrical properties of the proteins. Every attempt to explain the phenomena observed ends with the question, How does a protein particle floating about, for example, in a dilute hydrochloric acid assume an electropositive charge when the acid contains, as we know, an equal number of positive H ions and negative Cl ions? It is evident that this is only possible when protein takes up more positive H ions than negative Cl ions, or, as it is ordinarily stated, when the protein is semi-permeable to ions. The same holds in the case of alkalies for the OH ions. Many cells seem to have this same power, and HöBER has rendered it probable that red blood-corpuscles become positive when treated with carbonic acid, because they become permeable for some of the negative ions which they contain and which leave the red blood-corpuscles, thereby allowing an excess of positive ions to remain behind.

OSTWALD was no doubt the first to try to discover in the semi-permeability for ions the cause of the electrical phenomena observed in animal cells, and this suspicion has recently attained a very considerable degree of probability. OKER-BLOM and later BERNSTEIN have further developed this idea for the electrical phenomena observed in muscle and nerve, and sought experimental evidence for its support. If we imagine the surface of the muscle fibril to be more permeable for the positive ions than for the negative ions contained in the muscle, then the muscle must carry a positive charge externally and a negative one within. When two electrodes are laid upon the surface of an uninjured muscle, points having a different electrical potential are not touched, and the muscle shows no current.

As soon, however, as the one electrode is placed upon an artificially produced cross-section of a muscle—in other words, along the contents of the fibrils—the well-known current of rest passes in the outer circuit toward the negative exposed portions of the muscle plasma. The experiments of BERNSTEIN have shown that this current follows very accurately the typical laws governing ionic concentration chains. The same holds for the current of rest in nerves. Stimulation of the nerve brings about the well-known phenomenon of negative variation, in that it alters the permeability for ions.

Similar phenomena are observed in the electric organ of the torpedo, which has been studied by BERNSTEIN and TSCHERMAK. This organ consists of numerous plate-like cells arranged upon each other in a way similar to the plates of a voltaic pile and possessing a nervous end brush upon one side only.

When through nervous stimulation this side becomes more permeable for negative ions, an electric shock is produced through summation of the charges of the single cells, the intensity of which does not need to exceed that of a muscle current. As measurements indicate, the production of electricity in the electric organ seems also to follow in the main the thermodynamic laws governing concentration chains.

Let us return once more to the current of rest in muscle, which we have attributed to the semi-permeability of the plasma membranes for ions. If we imagine the permeability of this plasma membrane to be altered through some agency that precipitates protein or causes it to go into solution, then we may expect parallel variations in the current of rest. When HÖBER dipped the surface of

freshly cut frog's muscles into salt solutions of various kinds and measured the current of rest, he found that the effects of the different salts in this regard arrange themselves into a table similar to that given above for the precipitation of electropositive protein. The sign indicating a precipitation corresponds with a reversal in the current of rest, while that indicating a solution with the normal current of rest.

The electrical behavior of proteins is of importance to the histologist also for a proper understanding of the important cellular reactions which take place in fixation and staining. In spite of the fact that all the different portions of the cell are exposed to the same action of the fixing-agent, be this an indifferent substance, such as alcohol, or one imposing a positive charge, such as a solution of an acid or a heavy metal, the separate constituents of the cell react differently toward acid and basic dyes. Through the investigations of BILTZ in particular, the identity of the process of dyeing and colloidal reactions seems to be well established, so that we may assume that different portions of a cell may show different electrical states when exposed to the same external conditions. We will carry this discussion no further, but will only draw attention to an observation which is intimately connected with our own. E. MAYR (Graz) has studied under BETHE's direction the influence of salts upon the fixation and precipitation of nervous tissue. These studies have shown that the effects of ions upon the preservation and staining qualities of nerve fibres arrange themselves in a way similar to the table given on page 147 for the precipitation of electronegative protein, while the order of the ions is just the reverse and corresponds, in

the main, with that for the precipitation of electropositive protein when the ions are arranged in the order in which they render visible certain elements of the ganglion cells, such as NISSL bodies and nucleoli.

But that an electrical difference exists between nuclear substance and cell protoplasm even in the living cell. is rendered probable through many facts. RALPH· LILLIE found a difference in the direction in which spermatozoa and cells rich in protoplasm move in the electric current, and MARTIN H. FISCHER and WOLFGANG OSTWALD have already tried to give a physico-chemical theory of fertilization. However imperfect these attempts must of necessity seem, the successful establishment of a certain parallelism between those factors which, on the one hand, bring about artificial parthenogenesis and, on the other, cause a precipitation of solid colloids is of permanent value. The similarity of the formation of the astrosphere about the spermatozoon which has entered an egg with certain precipitations produced in colloids has been repeatedly noticed by investigators.

But we will keep from entering fields which have as yet been but little opened experimentally, and in closing point out a relation which by itself is not without general interest and which may also be of service to the investigator.

Between the reactions of colloids which take place with an equalization of electrical differences and the reactions of the immune bodies there exists a relation which is as intimate as anything can be, an idea which has already been illustrated in another place. Moreover, a more than accidental similarity seems to exist between immune reactions and the changes which take place in the·process of fertilization.

We know that the spermatozoon reacts specifically with the egg, that this specificity is, however, not absolute, as shown by the production of bastards. This specificity can also be altered through different chemicals, as shown by the remarkable hybridization experiments of LOEB. We see further that the spermatozoon becomes immobilized within the egg, in that a kind of precipitation, the formation of the astrosphere, a characteristic morphological sign of fertilization, starts from the spermatozoon.

Let us compare with this picture such a process as the agglutination of bacteria through immune serum. Here also there exists a specificity which is, however, by no means absolute, and here also an immobilization in the serum of the mobile bacterium. According to the pleasing idea of PALTAUF, we are dealing in this case with the formation of a precipitate about the capsule of the bacterium, something similar, therefore, to the change which occurs about the spermatozoon which has penetrated an egg. The specific effects of the immune bodies can also be altered through chemicals.

They are always associated problems, therefore, which arise in this or the other illustration used, and they all spring from the manifold similarity which exists between the colloids of the organism within and without the cells and which is determined to so great a degree by the electrical properties of the colloids.

There can be little doubt that out of the study of the physico-chemical properties of the colloids there will spring a new bud of physical physiology in which the application of the modern teachings of electricity will play a primary rôle. The physiology which recognizes

in the neighboring sciences of physics and chemistry that profound revolutionizing influence of the newer electrical investigations, which do not stop before even the most sacred and fundamental conceptions of this subject, must consider it as a next most worthy task to guarantee itself its share in the new conquests of scientific knowledge.

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